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CONTENTS

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THE PATHOLOGY OF THE BONE MARROW IN PERNICIOUS ANEMIA *

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In spite of the enormous amount of attention that has been devoted to the subject, it must be admitted that very little is actually known about the pathogenesis of pernicious anemia. Cohnheim,[1] who, in 1876, was the first to describe the bone marrow, regarded the anemia as due to a primary disturbance of blood formation, and many authorities have since maintained the same point of view. Another conception of the disease, however, regards the bone marrow changes as of a compensatory nature, and as the result of an attempt on the part of the organism to make good the losses arising from excessive blood destruction. This theory has become the more generally accepted one, even though the evidence of increased blood destruction is not convincing. Recent investigations of pernicious anemia have been, for the most part, concerned with the cell types and chemical constituents of the peripheral blood, but there is reason to believe that in order to understand the pathology of this disease, and indeed that of the other diseases of the blood-forming organs, more attention must be paid to the bone marrow than is now customary among clinical hematologists. The present study is a contribution to the pathology of the bone marrow in pernicious anemia, and the observations indicate that the changes in the blood are largely the result, rather than the cause, of abnormal bone marrow function.

There are two main reasons why comparatively few attempts have been made to correlate the pathologic changes in the bone marrow

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in pernicious anemia with the findings in the peripheral blood and the clinical condition of the patient. The first is that lack of definite knowledge of the normal anatomy and physiology of the bone marrow has made the interpretation of pathologic changes in the bone marrow almost impossible. This difficulty appears to have been met, to at least a large degree, by the recent work of Sabin and her associates,[2] which clarifies the situation in the normal, and opens the way to the investigation of the abnormal. The second reason is that, with few exceptions, all the studies of the bone marrow in pernicious anemia have been made on material obtained postmortem, and the pathologic changes represent the most advanced stage of an exceedingly complex process. This difficulty can also be surmounted if specimens of bone marrow are obtained at different phases in the relapses and remissions that are so characteristic of the disease. Such material can be secured by means of biopsies on the tibial marrow, and this paper deals with the pathology of the bone marrow at various stages in the course of pernicious anemia.

Puncture of the shaft of the tibia and withdrawal of small amounts of bone marrow for microscopic examination is by no means a new operation. It was apparently first performed on man in 1908 by Ghedini of Genoa,[3] who described his results in more than twenty cases, and recommended the method as a diagnostic procedure. Caronia [4] made bacteriologic examinations of the bone marrow in children with measles, and Morris and Falconer [5] studied the smears of tibial bone marrow in various pathologic conditions. More important use of the method was made by Zadek,[6] who compared the findings in the bone marrow and peripheral blood in a series of cases of pernicious anemia. He observed that the bone marrow, which is red in periods of relapse, becomes yellow and fatty during periods of remission, and he showed that the megalocytosis, which characterizes the blood picture in the relapse, corresponds to an increase of megaloblasts in the bone marrow.

All the above authors, who have studied the bone marrow intravitam, have concerned themselves, wholly or in large part, with smears rather than with sections of fixed tissue. The examination of smears of bone marrow is, however, unsatisfactory, for they do not necessarily give the correct idea of the relative number of different cell types present (thus megaloblasts may be so firmly adherent to one another, that only a small proportion appears in the smear), and

they give no indication of the actual structure of the bone marrow. As more is learned about the normal physiology of blood formation and the liberation of cells into the peripheral vessels, it becomes apparent that these physiologic functions can often be interpreted by the study of the anatomy of the bone marrow. It is thus evident that the elucidation of the pathologic physiology of the diseases of the hematopoietic system will be considerably aided by a better knowledge of the structure of the bone marrow, which can be acquired only from the study of sections of fixed tissue. The number of cases of pernicious anemia reported in this paper is relatively few (seven cases), but they suffice to throw light on the subject, and may serve as a basis for further work. It is also hoped that this method of study, namely, the study of the histology of the bone marrow at different stages of a disease, may be useful in the investigation of other pathologic conditions of the blood-forming organs.

There are certain obvious limitations to the value of an examination of small pieces of bone marrow from the tibia in the interpretation of the pathology of an organ which is so widespread in its distribution. The most important are, first, that the marrow of a long bone like the tibia is not entirely homogenous in structure, so that the specimen obtained by puncture may not be representative of the marrow as a whole, and second, that the pathologic process in the marrow of the tibia does not necessarily correspond in extent and in degree to that in the bone marrow of other regions. These points are admirably brought out in the colored illustrations in Sheard's [7] book, which show the variations in gross appearance of the marrow in different bones from a case of pernicious anemia. The evidence which has accumulated in this laboratory, from a study of bone marrow obtained intravitam and postmortem in a much larger group of cases of pernicious anemia than is reported in the present paper, seems to indicate that the pathologic process usually starts in the active marrow of the flat bones and vertebrae, and while progressing in them, spreads peripherally so that it involves the femur and later the tibia. Thus the process in the tibia is often less advanced than in the femur. Presumably the same course of extension to the periphery goes on in the arms. Within an individual long bone, the process generally begins at the epiphysis and spreads gradually to the center of the diaphysis. With the retrogression of the pathologic changes, during a clinical remission, the process is usually

reversed, and begins to clear up first in the tibia. Thus the marrow of the tibia, which may be involved only rather late, is, nevertheless, a more sensitive index of the extent and degree of the pathologic process than is, for instance, the marrow of the femur. Bearing in mind all the limitations that one must accept in examining small specimens of marrow from the tibia, there yet remains much to be learned from them, and this is especially true when two or more specimens can be obtained from a single case at different periods in the course of the disease.

Technic of Tibial Puncture

The operation of tibial puncture must, of course, be carried out under the strictest surgical precautions. I am greatly indebted to Dr. Robert C. Cochrane for all the specimens of bone marrow obtained during life. The soft tissues over the middle of the anterior or mesial aspect of the tibia were carefully infiltrated with a 2 per cent novocain solution over an area sufficient to permit a longitudinal incision about 4 cm. in length down to the periosteum. After elevation of the periosteum, the marrow cavity was entered by means of a small drill or a trephine which removed a 6 mm. cylinder of bone. Then small pieces of the marrow, 1 to 3 mm. in diameter, were removed by means of a small sharp bone curette, the cavity of which was rather well recessed and straight-sided. The specimens were immediately fixed in Zenker's solution, later embedded in paraffin, sectioned, and stained with eosin and methylene blue. Hemorrhage into the tissues sometimes complicated the interpretation of the histology. In sewing up the wound it was found best to use no sutures except in the skin, and to apply a tight bandage to prevent oozing. No untoward results have been observed in a series of eighteen operations, and the fact that the discomfort to the patient was not great is indicated by there having been two tibial punctures on several patients and three punctures on one patient. It is appropriate, however, to express deep appreciation for the coöperation of the patients who willingly submitted to these operations.

The description of the clinical cases and the observations on the condition of the bone marrow follow. The nomenclature of cells in the bone marrow is according to the terminology established by Sabin.[2]

CASE 1. A. F., a man 33 years old, with a history typical of pernicious anemia extending over two years, and apparently in his third relapse. Physical examination, laboratory tests and hematologic studies were all characteristic of the disease.

May 18, 1925, examinations of blood showed: hemoglobin, 15 per cent; red blood cells, 0.6 million per c.mm.; leucocytes, 5400 per c.mm.; serum bilirubin, 1.5 mg. per 100 cc.

Biopsy of tibial marrow, May 19, 1925. The tissue was very cellular, and the fat, which is normally present in the marrow of the tibia, had been completely replaced by cells. The histologic picture was as complex as that of the bone marrow obtained postmortem in pernicious anemia, and was, in general, similar to it in character. The most striking feature was the enormous hyperplasia of megaloblasts. These cells, which have large vesicular nuclei with a definite chromatin network, and basophilic cytoplasm, were found in clumps, cords and columns, and sometimes as separate cells. Some of this separation of individual cells was unquestionably due to shrinkage in the process of fixation. For the most part, the megaloblasts were adherent to one another and the appearance of the tissue was suggestive of a tumor. Rapid multiplication of megaloblasts was indicated by the great number of mitoses, sometimes as many as six or eight being found in a single field with the oil immersion lens. Fig. 1 is a photograph under low power to show the general character of the tissue and the distribution of the megaloblastic hyperplasia. Fig. 2 is a photograph under high magnification, and Figs. 3 and 4 are drawings to illustrate the megaloblasts and their mitoses. In addition to the megaloblasts, the marrow contained many normoblasts and cells intermediary between megaloblasts and normoblasts. There were relatively few mature erythrocytes. Giant cells were present in moderate number, while myelocytes and leucocytes were fairly common. A few myelocytes were found in mitosis. In contrast to the usual findings in bone marrow obtained postmortem from cases of pernicious anemia, there was scarcely any phagocytosis of red blood cells by clasmatocytes.[8] The venous sinusoids, which are frequently such a prominent feature in early or slight degrees of bone marrow hyperplasia, were narrow, compressed and recognized only with great difficulty.

Two months later, on July 20, 1925, the blood examinations were as follows: hemoglobin, 13 per cent; red blood cells, 0.8 million per

c.mm.; leucocytes, 1200 per c.mm.; serum bilirubin, 0.57 mg. per 100 cc. On July 22, a second tibial puncture was performed, and it is of interest, that shortly afterwards a spontaneous remission set in.

Biopsy of tibial marrow, July 22, 1925. The tissue was very similar to that obtained on May 18, but there were occasional cells containing globules of fat, and there was a definite increase in the number of normoblasts and mature red blood corpuscles. The megaloblastic hyperplasia was still the predominant feature, however, and there were many mitoses of megaloblasts. There were also more cells of the leucocyte series. The venous sinusoids remained compressed and obscure.

On August 7, the hemoglobin was 34 per cent, and the erythrocyte count was 1.5 million per c.mm. On September 2, "great clinical improvement" was noted. On September 28, the hemoglobin was 52 per cent and the red blood cell count was 2.4 million per c.mm. During the following months the patient lived on a diet containing large amounts of liver, and a striking clinical remission took place. On March 12, 1926, the blood examinations were as follows: hemoglobin, 86 per cent; red blood cell count, 5.5 million per c.mm.; leucocytes, 6200 per c.mm.

Biopsy of tibial marrow, March 12, 1926. A large part of the specimen was composed of fat cells, and was without evidence of myeloid hyperplasia. The intersinusoidal capillaries in this area were filled with normal erythrocytes, but it was impossible to determine whether this was a true hyperemia or was the result of hemorrhage during the operation. Another part of the specimen consisted of well filled fat cells, separated from one another by small groups of myeloid cells which apparently were within intersinusoidal capillaries. The capillary endothelium was moderately hypertrophic and hyperplastic. Within the capillaries were a few megaloblasts and erythroblasts, very many normoblasts and many mature erythrocytes. There was also a moderate number of cells of the leucocyte series. The venous sinusoids were widely distended with blood, and the conical openings of the intersinusoidal capillaries into them were easily made out in many places. The whole picture resembled that of the early stage of simple marrow hyperplasia.[9] Fig. 5 shows the general character of the more cellular part of the specimen, and Fig. 7 shows the predominance of normoblasts in the islands of myeloid cells.

Summary of findings in Case 1

At the height of a severe relapse (May 19, 1925), the marrow was characterized chiefly by the complete replacement of fat by myeloid cells, and by the great hyperplasia of megaloblastic tissue with numerous mitoses. There were many normoblasts, but relatively few mature erythrocytes, and the venous sinusoids were narrow and compressed. Two months later (July 22, 1925), just before the onset of a clinical remission, the marrow was similar except for the presence of a few cells containing fat, and a relative increase of normoblasts and mature red blood cells. Ten months after the first examination (March 12, 1926), during a remission in which the erythrocyte count was normal, the cellular hyperplasia had almost completely disappeared and the marrow consisted largely of fat cells. In the small capillary spaces, between some of the fat cells, were many erythrocytes and normoblasts, but the more primitive cells (megaloblasts) were comparatively few in number. The venous sinusoids had become widely distended with blood.

CASE 2. C. H., a woman 46 years of age, who had been under observation for four years as a typical case of pernicious anemia. There had been several relapses followed by periods of moderate remission. On March 12, 1926, she was in the hospital during a severe relapse and the blood examinations were as follows: hemoglobin, 24 per cent; erythrocytes, 0.9 million per c.mm.; leucocytes, 4400 per c.mm.; serum bilirubin, 0.9 mg. per 100 cc.

Biopsy of tibial marrow, March 12, 1926. The tissue showed a great hyperplasia of myeloid cells and relatively few cells containing fat globules. Fig. 8 is a photograph to show the general character of the tissue, and the displacement of the fat. The histology resembled that seen in Case 1 during the relapse. There was a striking hyperplasia of megaloblasts with a general tendency on the part of the cells to adhere to one another and to lie in clumps and columns. Rapid cell growth was indicated by the many mitoses seen in the megaloblasts. There were many normoblasts, but few mature red blood cells, few giant cells and few leucocytes. The venous sinusoids were often outlined by pigment granules in the endothelium, but they were compressed and indistinct. Abnormal phagocytosis of erythrocytes or of pigment was not observed.

Immediately after the above observation was made, the patient

began to live on a diet containing much liver, and a prompt clinical remission set in with rapid rise in the red blood corpuscles. On April 29, 1926, the erythrocytes had risen to 3.5 million per c.mm., and a second biopsy was performed on the tibial marrow. The other blood examinations showed: leucocytes, 8500 per c.mm.; serum bilirubin, 0.18 mg. per 100 cc.

Biopsy of tibial marrow, April 29, 1926. The tissue consisted largely of cells well filled with fat. It had considerably more fat than has normal vertebral marrow. Fig. 6 is a photograph, under low magnification, to show the relation of fat to cellular areas (compare with Fig. 8). The small cellular areas between the fat globules were chiefly composed of erythrocytes in the intersinusoidal capillaries, but there were also a great many normoblasts, some of which showed definite mitoses. The normoblasts were often in large clumps which filled the spaces between the fat cells, as shown in Fig. 9. Megaloblasts were not a prominent feature but there were a few small groups and a few scattered single cells. Mitosis were rare among the megaloblasts. Leucocytes and giant cells were few in number. The venous sinusoids were outlined by pigment in the endothelium, and they were comparatively wide and distinct.

Two months later, on June 29, 1926, the hemoglobin had risen to 92 per cent; and the erythrocyte count to 4.9 million per c.mm.

Summary of findings in Case 2

During a severe relapse (March 12, 1925), the fat of the bone marrow was almost entirely replaced by myeloid cells, and there was a striking hyperplasia of megaloblasts with many showing mitoses. Many normoblasts were present, but mature red blood cells were not particularly numerous. Early in the development of a rapid remission (April 29, 1926), the bone marrow was characterized by a great increase in fat deposits and by large numbers of normoblasts and mature erythrocytes, but at this time, only a few megaloblasts were observed. The venous sinusoids were much more distended and distinct during the remission than during the relapse.

CASE 3. V. D., a man 48 years old, with symptoms of anemia for one year, together with the physical and laboratory findings typical of pernicious anemia. On July 15, 1925, the hemoglobin was 43 per cent, and the red blood cell count 2.4 million per c.mm. On August

20, the hemoglobin was 31 per cent, and the red blood cell count 1.0 million per c.mm. On September 2, the blood examinations showed an erythrocyte count of 1.1 million per c.mm.; leucocytes, 4100 per c.mm.; reticulocytes, 1.1 per cent; and the serum bilirubin, 0.8 mg. per 100 cc. The patient was thus having a relapse.

Biopsy of tibial marrow, September 2, 1925. The tissue contained a considerable amount of fat, about the amount found in normal vertebral marrow. The general character is shown in Fig. 11. There were many rather large islands of megaloblasts arranged in clumps or columns, and many single megaloblasts. Megaloblasts and erythroblasts were frequently in close relation to the fat cells, and their position suggested that they arose from the endothelium lining the intersinusoidal capillary spaces. Mitoses of megaloblasts were found in moderate numbers. There were very many normoblasts, and several definite mitoses were observed among them. Mature erythrocytes were present in moderate numbers. Phagocytosis of red cells was not seen. Giant cells were rare, and there were few myelocytes or leucocytes. The sinusoids were compressed and indistinct.

In the subsequent months there was no striking change in the patient's condition, but he gradually failed in health. On Jan. 18, 1926, the blood examinations were as follows: hemoglobin, 24 per cent; erythrocytes, 0.9 million per c.mm.; leucocytes, 3500 per c.mm.; serum bilirubin, 0.66 mg. per 100 cc. On this day a second tibial biopsy was performed. The patient left the hospital soon after, and died at home on Feb. 26, 1926. The second biopsy was thus made almost at the end of the terminal relapse.

Biopsy of tibial marrow, January 18, 1926. The tissue contained about as much fat as was present in the first biopsy. There was a striking hyperplasia of the megaloblasts, rather more than in the first biopsy, the cells lying in clumps which were so large that they sometimes filled the spaces between the fat cells. Fig. 10 shows the amount of fat present and the extensive hyperplasia of megaloblasts. Mitoses were common among the megaloblasts, as many as five having been observed in a single field of the oil immersion lens. There were very many normoblasts but comparatively few mature red blood cells. For the rest, the tissue was similar to that observed on September 2.

Summary of findings in Case 3

Two specimens of tibial marrow were obtained during the course of the prolonged terminal relapse, the first about six months and the second about one month before death. The two specimens resembled each other closely and both contained a considerable amount of fat. In this connection it may be noted, however, that sections from other cases show that even at death the replacement of fat by myeloid hyperplasia is frequently much less complete in the tibia than in the femur. The marrow from both biopsies showed great hyperplasia of megaloblasts, but this process was somewhat more extensive in the second specimen. Normoblasts were present in great numbers, but there were comparatively few mature erythrocytes in either piece of tissue.

CASE 4. B. C., a woman who gave her age as 48 years, but who appeared to be considerably older. She had been "weak" for four months, and reported having numbness of the fingers for one month. Physical examination and laboratory tests were typical of pernicious anemia. Blood examinations were as follows: Aug. 1, 1925, hemoglobin, 18 per cent; erythrocytes, 0.8 million per c.mm.; leucocytes, 5500 per c.mm.; August 20, hemoglobin, 25 per cent; erythrocytes, 1.3 million per c.mm.; leucocytes, 4800 per c.mm.; August 31, hemoglobin, 42 per cent; erythrocytes, 2.3 million per c.mm.; leucocytes, 6900 per c.mm.; September 30, hemoglobin, 63 per cent; erythrocytes, 3.5 million per c.mm.; leucocytes, 8100 per c.mm. A biopsy performed on September 2 was, therefore, done at a time when the patient was making rapid spontaneous improvement.

Biopsy of tibial marrow, September 2, 1925. The fat of the marrow had been entirely displaced and the tissue had a solid appearance. Mature red blood cells were the most prominent feature, and it is possible that they may have been due to hemorrhage, but their relation to the islands of myeloid tissue suggests that they were true components of the marrow. Megaloblasts were found in a limited number of small clumps and as scattered individual cells. Mitosis of megaloblasts was infrequent. There were a great many normoblasts in large and small groups. Giant cells were few in number, and leucocytes were fairly numerous. The sinusoids were compressed.

On Oct. 27, 1925, the blood examinations were as follows: hemoglobin, 71 per cent; red blood cells, 3.5 million per c.mm.; leucocytes, 9100 per c.mm. The second biopsy was performed on Oct. 28, 1925, at a time of marked clinical improvement, and just before the patient left the hospital. She was then in a rather prolonged remission of moderate degree. The patient was subsequently fed on liberal amounts of liver, and about eight months later the erythrocyte count was 4.6 million per c.mm.

Biopsy of tibial marrow, October 28, 1925. This tissue was taken from somewhat higher up in the tibia than was the first specimen. It contained about as much fat as normal vertebral marrow. There were a few small clumps of megaloblasts and scattered individual cells, but they were not a prominent feature. Mitoses of megaloblasts were found rarely. There were many normoblasts, but only a moderate number of mature erythrocytes. Cells of the leucocyte series were very numerous and giant cells were not uncommon. The sinusoids were somewhat more distinct than in the tissue from the former biopsy. The appearance of the tissue resembled that of normal active marrow from a vertebra, except that there were rather more megaloblasts.

Summary of findings in Case 4

The tissue obtained on Sept. 2, 1925, soon after the onset of a spontaneous improvement, contained no fat, and the cells consisted largely of erythrocytes and normoblasts. Megaloblastic hyperplasia was not a prominent feature. The specimen is to be compared with that of Case 2, taken on April 29, 1925, which was also obtained during a remission, and which differs from Case 4 chiefly in that it contained more fat. Both sections showed a predominance of normoblasts and mature red blood cells, and in both the megaloblastic hyperplasia was relatively slight. Two months later (Oct. 29, 1925), after the patient's still further improvement, the bone marrow showed an increase of fat, and a cell picture which resembled that of normal active vertebral marrow except for the moderate increase of megaloblasts. There were no longer such large numbers of erythrocytes in the marrow and it is probable that they had passed out into the capillaries. It may also be that after the relapse was over, the part of the marrow near the epiphysis (this specimen was taken from high up on the tibia) continued to function

as normal marrow. Unfortunately no specimen was obtained during the more complete remission that took place eight months later.

CASE 5. J. S., a man 61 years old, who had run a characteristic course of pernicious anemia for about eighteen months, most of which time he had been under observation. In August, 1925, he entered the hospital, shortly after the onset of his second relapse, with physical examination and laboratory tests wholly typical of pernicious anemia. The results of blood examinations were as follows: March 26, 1925, hemoglobin, 72 per cent; erythrocytes, 3.9 million per c.mm.; leucocytes, 8200 per c.mm.; June 12, 1925, hemoglobin, 82 per cent; erythrocytes, 3.3 million per c.mm.; leucocytes, 5800 per c.mm.; Aug. 25, 1925, hemoglobin, 30 per cent; erythrocytes, 1.3 million per c.mm.; leucocytes, 5500 per c.mm.; Sept. 2, 1925, erythrocytes, 1.3 million per c.mm.; leucocytes, 4900 per c.mm. During these months he was, therefore, slowly going into a severe relapse.

Biopsy of tibial marrow, September 2, 1925. A part of the section consisted of fat cells with mature erythrocytes filling the intersinusoidal capillary spaces (hemorrhages?). The rest of the section showed much hyperplasia of myeloid cells between the fat cells, (there was about as much fat here as in normal vertebral marrow) and in one area the fat was completely displaced. The hyperplastic areas were composed chiefly of megaloblasts, growing in columns and islands, and often filling the space between the fat cells. There were many mitoses of megaloblasts. Fig. 12 shows a clump of megaloblasts between fat cells and one megaloblast undergoing division. Just below and to the left, there is another megaloblast in the same phase of mitosis, but it does not show clearly in this focus. There were a great many normoblasts, a moderate number of mature red blood cells, and many cells of the leucocyte series. The sinusoids were compressed and indistinct.

In the subsequent weeks the patient continued to fail gradually. The blood examinations on Dec. 7, 1925, were as follows: erythrocytes, 0.7 million per c.mm.; leucocytes, 9200 per c.mm.; serum bilirubin, 1.21 mg. per 100 cc. He died on the same day.

Tibial marrow at necropsy (two hours after death). The tissue showed the typical, extremely confused structure usually found at necropsy. The fat had been almost entirely replaced by myeloid

cells. The prominent feature was the hyperplasia of megaloblasts, the cells being single, in clumps, or in columns. There were many mitoses among the megaloblasts. Normoblasts were common. Giant cells were rare. There were many myelocytes and leucocytes. Throughout the tissue, and so numerous that there were often six or eight in a high power field, were clasmatocytes (endothelial cells) which had phagocyted erythrocytes, normoblasts, and occasionally leucocytes. The number of ingested red blood cells was enormous. The red cells within the phagocytes usually retained their normal appearance, and there were few phagocytes containing hemosiderin. Some of the sinusoids were broad and well defined, but most of them were compressed and difficult to distinguish.

Summary of findings in Case 5

On Sept. 2, 1925, during a severe relapse which took place three months before death, the bone marrow contained a considerable amount of fat, but between the fat cells there was an active hyperplasia of megaloblasts, with many normoblasts. On Dec. 7, 1925, the bone marrow, taken two hours after death, showed such an extensive increase in myeloid cells that the fat had almost completely disappeared. Hyperplasia of megaloblasts was the predominant feature, but there were many normoblasts and cells of the leucocyte series. Of considerable interest was the appearance of great numbers of clasmatocytes which had phagocyted erythrocytes and normoblasts.

In addition to the above five cases in which two or more specimens of bone marrow have been obtained at different times, brief mention will be made of two additional cases in which only one specimen has been obtained, but from which, nevertheless, certain impressions may be formed.

CASE 6. M. H., a man 55 years old, came under observation in the first relapse of typical pernicious anemia. The symptoms had lasted about six months. Blood examinations on Jan. 25, 1926, were as follows: hemoglobin, 50 per cent; erythrocytes, 1.3 million per c.mm.; leucocytes, 2040 per c.mm.; reticulocytes, 1.6 per cent. Liver feeding was begun on January 28, and on February 12 the blood examinations showed: hemoglobin, 50 per cent; erythrocytes, 1.8 million per c.mm.; leucocytes, 3900 per c.mm.; reticulocytes, 9.1 per cent.

Biopsy of tibial marrow, February 12, 1926. The specimen consisted of fat tissue and showed no evidence of increased vascularity or cellular hyperplasia. It was normal, atrophic, fatty marrow.

Summary of findings in Case 6

This specimen was taken shortly after the onset of what subsequently proved to be a very rapid remission following a first and relatively short relapse. If this sample represents the general character of the tibial marrow, then the presence of a normal fatty marrow can be explained either by the fact that, at least in the first relapse, the pathologic process does not necessarily extend to the marrow of the tibia, or by the assumption that the pathologic process can disappear completely, and very rapidly, during a period of clinical improvement.

CASE 7. A. H., a woman 64 years old, with a history of pernicious anemia of two years duration. She entered the hospital during a relapse with an erythrocyte count of about 1.0 million per c.mm. For three weeks she ate considerable amounts of liver, and at the end of this time (May 26, 1926) the blood examinations were as follows: hemoglobin, 57 per cent; erythrocytes, 2.6 million per c.mm.; leucocytes, 6000 per c.mm.; reticulocytes, 4.4 per cent.

Biopsy of tibial marrow, May 26, 1926. The specimen showed essentially a fatty, normal, aplastic, tibial marrow. There were a few rather large endothelial cells with vesicular nuclei, lying between the fat cells and forming the walls of intersinusoidal capillaries. These resemble the hypertrophied endothelial cells which are characteristic of the earliest stage of marrow hyperplasia,[9] but they were so few in number that it is impossible to say that they were not within the normal limits.

Summary of findings in Case 7

In this case, as in Case 6, it is unfortunate that no biopsy was performed before the onset of the remission. Here again, it cannot be determined whether the normal appearing marrow was the result of a rapid clearing up of the pathologic process after the onset of a remission, or whether it merely indicated that even after the disease had lasted two years, the tibial marrow had not become involved. The fact that in Case 2 a remission of even greater degree and

rapidity was accompanied by a striking change, but not by a disappearance of the pathologic process, may be taken to suggest that in Cases 6 and 7 the marrow of the tibia had never been affected.

Discussion

The essential lesion of the bone marrow in pernicious anemia, and that which dominates the histologic picture during clinical relapse, is an hyperplasia of the myeloid cells in which the megaloblasts play the chief part. The development of the process can be studied in a simple fatty marrow like that of the tibia more easily than in complex active marrow like that of the vertebrae, but the lesion seems to be the same wherever it occurs. The megaloblasts develop from the endothelial cells of the intersinusoidal capillaries which, in an a atrophic marrow, are collapsed and almost invisible between the fat cells.[2, 9] They are formed within the lumen of the capillary, and where active proliferation is taking place, the capillary may be entirely filled by one, two or more rows of megaloblasts. This is illustrated in Fig. 12. In the more active stages of the pathologic process, as seen at necropsy, and in tissue taken at biopsy during a relapse, the proliferation of megaloblasts is very rapid. This is indicated by the extraordinary number of mitoses, and by the tendency of the cells to remain adherent to one another in columns and clumps, rather than to separate off as individual cells. Coincident with the hyperplasia of megaloblasts there is also a limited development of the more highly differentiated forms of the red blood cell series, namely erythroblasts, normoblasts, and erythrocytes. These cells also proliferate in the capillary spaces between the fat cells, and, since the marrow is confined within a rigid shaft of bone, their multiplication goes hand in hand with a disappearance of the globules of fat. It is evident, however, that even after the globules of fat are displaced, the fat cells remain in their normal position in the marrow, for specimens taken at biopsy show that when the myeloid hyperplasia recedes, the fat cells take up globules of fat again, and fill the space of the marrow cavity. The fat cells are practically invisible during the period of myeloid hyperplasia, but they are a constant element in the structure of the marrow, and serve an important subsidiary function by compensating for the proliferation and retrogression of the true blood-forming cells. The number and types of leucocytes vary in the bone marrow in pernicious anemia,

but many are found in the stages of most active hyperplasia, while giant cells are almost always abnormally decreased.

The study of bone marrow obtained by biopsy at different stages of pernicious anemia throws light on the relation of the pathologic process in the bone marrow to the clinical course of the disease, and the observation of Zadek [6] that the marrow hyperplasia disappears during clinical remissions has been confirmed by this investigation. In general, the more active the disease and the more profound the relapse, the greater is the pathologic hyperplasia of the bone marrow. This is, to some extent, indicated by the relation of cellular hyperplasia to the amount of fat in the marrow. Thus in the terminal stage, as shown in tissue obtained at necropsy, there is usually a complete or nearly complete replacement of fat by myeloid hyperplasia (see, for instance, Case 5). A similar but sometimes less marked condition is found during a serious relapse. In a severe relapse, Case 1 showed complete disappearance of fat, and the marrow in Case 2 contained extremely little fat. On the other hand, considerable fat may be present at autopsy in the marrow of a peripheral bone like the tibia, and in Case 5, the marrow contained a good deal of fat during the progress of the terminal relapse. The first specimen in Case 4, obtained after the onset of a remission, contained no fat. The relationship is thus by no means constant, and the displacement of fat, although an index of the cellularity of bone marrow, does not necessarily run parallel to the clinical course of the disease.

Of much greater significance in relation to the clinical course of the disease than either the amount of fat or the degree of cellularity of the bone marrow are the types of cells of which the marrow hyperplasia is composed. Thus the evidence indicates that severe relapses are characterized by a predominance of rapidly proliferating megaloblasts, while in remissions or during periods of clinical improvement the megaloblastic hyperplasia becomes less evident, and more mature cells of the red blood cell series, normoblasts and erythrocytes, become the prominent feature in the marrow. Essentially the same observations were made by Zadek.[6] Cases 1 and 2 show the change in cell type very clearly, for specimens of bone marrow were obtained, first in relapse and then at the height of a remission, or during the development of it. The first specimen from Case 4, taken soon after the onset of a remission, showed many normoblasts and erythrocytes,

but comparatively few megaloblasts. In Case 3, on the other hand, there was a slight increase in megaloblastic hyperplasia as the relapse progressed, and in Case 5 the marked hyperplasia of megaloblasts, seen during the course of the terminal relapse, was found to be still further increased at necropsy. Although the megaloblastic hyperplasia seems to be the essential feature of the pathology of the bone marrow in pernicious anemia, it cannot be stated that the lesion is necessarily specific for this disease.

Cases 6 and 7, in which normal, fatty marrows were obtained early in the development of clinical remissions, suggest, without definite proof, that pernicious anemia may exist for a considerable time and even present the picture of a serious relapse, without involvement of the marrow of the tibia. It is quite possible that for indefinite periods the disease may be limited to those parts of the marrow that are normally active. At necropsy an involvement of the marrow of the femur is, of course, practically constant.

The study of specimens of bone marrow taken at different stages in the course of pernicious anemia also furnishes evidence on the long disputed question of whether the anemia is primarily due to an increased destruction of red corpuscles, or whether it is the result of a primary disorder of blood formation in the bone marrow. At present the most widespread opinion appears to be that the disease is a hemolytic type of anemia, and that the bone marrow undergoes a compensatory hyperplasia as the result of the blood destruction. The histology of the marrow, however, does not tend to support this theory. The evidence of Zadek,[6] together with that presented above, shows that the characteristic megaloblastic hyperplasia is most highly developed in severe relapses, and disappears, completely or in large part, during the remissions. This, in itself, might be interpreted as meaning that the hyperplasia recedes as soon as the hemolytic process ceases. It is, therefore, significant to observe further that the megaloblastic hyperplasia begins to decrease, and the cytology of the marrow becomes more normal, very early in the development of a clinical and hematologic remission, and at just the period when one might expect a compensatory hyperplasia to be most marked. In Case 1, just before a remission started, there was a slight increase of fat (indicating a less cellular marrow) with an increase of cells more mature than megaloblasts, and after a complete remission had taken place the marrow showed only slight signs of

the previous hyperplasia. In Case 2, there was a striking decrease in megaloblastic hyperplasia early in the course of a rapidly developing remission. In Case 4, the marrow taken soon after the onset of a remission showed little megaloblastic hyperplasia. On the other hand, Case 5 illustrates that as a relapse progresses the opposite condition will be found, namely that the megaloblastic hyperplasia increases.

Such histologic evidence, however, does not prove that the pathologic condition of the marrow is the cause of the anemia, in spite of the fact that it suggests that the decrease in megaloblastic hyperplasia precedes the improvement in the hematologic picture. It is, therefore, interesting to correlate what is known about the pathologic histology of the marrow with some of the characteristic changes of the red blood corpuscles in the peripheral blood. In a severe relapse, with an erythrocyte count of 1.0 million or less per c.mm., the number of young cells in the blood, as shown by a count of reticulocytes, is usually relatively increased, but absolutely normal or decreased. Thus a reticulocyte count of 2 per cent of the total red blood cells, which is common under such circumstances, actually means that no more young cells are being put out by the bone marrow per day than is the case in a normal person with a red blood cell count of 5.0 million per c.mm. and approximately 0.5 per cent reticulocytes. This is so in spite of the fact that the active bone marrow, in the patient with a relapse of pernicious anemia, is an organ many times larger than that in the normal subject. The extensive hyperplastic marrow delivers fewer young cells in a unit of time than a normal marrow. There is cellular hyperplasia with functional inefficiency. This becomes particularly clear if one compares the situation with that in congenital hemolytic jaundice, a disease which is probably of a primary hemolytic nature. Here the marrow continues for months and years to put out so many young cells that the percentage of reticulocytes may be 15 to 30 or more of a red blood cell count between 4.0 and 5.0 million per c.mm. In addition to this, it is during the development of a remission that one often finds the large numbers of reticulocytes in the peripheral blood in pernicious anemia, and it has been seen that at exactly this period, when the bone marrow is beginning to function more effectively, the megaloblastic hyperplasia is beginning to disappear. Such histologic and hematologic evidence, therefore, indicates that

the megaloblastic hyperplasia of pernicious anemia produces a bone marrow with diminished functional capacity, and it leads to the belief that this type of anemia is the result of the pathologic lesion in the bone marrow.

The histologic material now at hand, can only suggest why it is that the megaloblastic hyperplasia of pernicious anemia produces a bone marrow of diminished functional efficiency. At the height of a relapse, when the output of cells from the marrow is at its lowest, there is an extraordinarily rapid and extensive proliferation of megaloblasts, but the relative number of more mature cells in the bone marrow, normoblasts and erythrocytes, is usually diminished. During the progress of a remission on the other hand, when the marrow is hyperactive, there are fewer megaloblasts, but many more normoblasts and erythrocytes. The relapse is thus characterized by the rapid proliferation of primitive cells, and by a diminished tendency towards the differentiation of the more mature forms of the erythrocyte series, while the onset of a remission is marked by a resumption of a more normal process of cell differentiation. The cause of the anemia would thus appear to be an abnormal type of cell growth consisting in a development of the primitive megaloblasts, and a failure of differentiation of the more mature red blood cells that normally get into the peripheral blood. There is little to indicate whether this is to be regarded as a hyperplasia due to some extraneous toxin, or whether the process is similar to that of a tumor growth. The extraordinary clinical results that have been obtained recently in the production of remissions in pernicious anemia by the feeding of large amounts of liver [10] suggest that this organ possesses some factor which affects cellular metabolism, and promotes the differentiation of the more mature cell types.

In addition to the above, it is worth noting that the venous sinusoids, into which mature erythrocytes are normally discharged, are extremely narrow and compressed in specimens of highly cellular marrow, and it is conceivable that a decrease of the vascular bed is a secondary factor in preventing red blood cells from getting out of the marrow.

It is also worthy of note that the phagocytosis of erythrocytes, which is such a striking feature in the bone marrow obtained at necropsy in almost all cases of pernicious anemia (see Case 5, bone marrow at necropsy), has rarely been observed in tissue obtained at

biopsy, even when this was taken during a severe relapse. The most obvious explanation is that the phenomenon occurs only postmortem, but there are several points that cast doubt on such an hypothesis. Thus it must be remembered that while phagocytosis of red blood cells may be found in any type of marrow at necropsy, it is particularly constant and prominent in pernicious anemia. In addition to this, it has often been found in bone marrow 1 to 2 hours after death (the necropsy in Case 5 was performed 2 hours after death), and shows no tendency to be more marked if the autopsy is performed later. The question could be settled by the examination of tissue taken a few hours or days before death, but such material is not at hand. The phagocytic cells are clasmatocytes and, according to Sabin, they are derived from endothelial cells. In marrows obtained at biopsy, it is extremely hard to distinguish clasmatocytes from endothelial cells as they are obscured by the confused mass of myeloid cells (just as fat cells may be invisible in a very hyperplastic marrow), but when they have ingested erythrocytes and normoblasts, the clasmatocytes become enlarged and are easily seen. It is possible that clasmatocytes are actually increased in number from an early stage in the disease, but that they become phagocytic only in the terminal stage. If this is shown to be true it would be a fact of considerable importance, for it would indicate that the pathology of pernicious anemia is characterized by the proliferation of two derivatives of the endothelial cell, the megaloblast and the clasmatocyte, and it might be possible to go back one step further and consider whether the primary lesion is not associated with the endothelial cells. Rich [11] showed that clasmatocytes, grown *in vitro*, ingest red blood corpuscles with which they are brought in contact. Contact between clasmatocytes and erythrocytes seems to lead to phagocytosis. Is it possible that in the terminal stage of pernicious anemia the red blood corpuscles are not delivered to the circulation, but remain in the marrow, where they come in contact with large numbers of clasmatocytes by which they are ingested?

The fact that megaloblasts are not frequently found in the peripheral blood, even during a relapse when they are numerous in the bone marrow, and the comparative rarity of other immature forms in the blood stream, is best explained by Key's [12] observation that immature red blood cells tend to adhere to one another. Under such

circumstances they would not be easily displaced from the marrow into the circulating blood.

If pernicious anemia be considered as primarily due to a bone marrow lesion rather than to a hemolytic process, the question naturally arises as to how one can explain the excess of bilirubin which is found in the blood plasma. In the absence of more definite knowledge of the physiology of pigment metabolism, one can only suggest that it results from an excess of pigment over and above what the marrow can use in constructing erythrocytes. This is consistent with the fact that bilirubin is increased during relapse, when the marrow is inefficient, and falls soon after the onset of a remission. It is also in harmony with the conception of Whipple [13] who regards the disease as being due to the decreased formation of the stroma of red blood cells, rather than to the lack of the constituents of hemoglobin. The erythrocytes in pernicious anemia are, indeed, more than normally filled with hemoglobin. Reference may also be made again to that most definite type of hemolytic disease, congenital hemolytic jaundice, in which the amount of bilirubin in the plasma is many times greater than it is in pernicious anemia.

Conclusions

1. Observations on the structure of the bone marrow in pernicious anemia, made on tissue obtained at biopsy at different stages of the disease, show that the myeloid hyperplasia is most marked during relapse, and that the structure of the marrow tends to return to normal during remission.

2. During relapse the essential histologic lesion is a rapid and extensive proliferation of primitive cells (megaloblasts), with a relatively diminished tendency towards the differentiation of mature cells of the erythrocyte series. The bone marrow shows a cellular hyperplasia, but it is functionally inefficient.

3. Remissions are characterized by the presence of few megaloblasts and a great relative increase of normoblasts and mature red blood cells in the bone marrow.

4. The anemia of the relapse is explained by the functional ineffectiveness of the bone marrow, which results from the failure of the megaloblasts to differentiate towards mature erythrocytes. The blood picture of the remission is explained by the resumption of a

more normal type of cell development with an increased production of normoblasts and erythrocytes.

5. It is suggested that the striking clinical results obtained by the feeding of large amounts of liver in the production of prompt and marked remissions may be due to some factor in the liver which affects cell metabolism and promotes the development and differentiation of mature red blood cells.

I am greatly indebted to Dr. W. R. Castle for assistance in obtaining the pathologic material, to Miss E. Piotti for the drawings, and to Dr. Henry Jackson, Jr. for the photomicrographs.

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DESCRIPTION OF PLATES

Plate 57

Fig. 1. Case 1. May 19, 1925. General character of marrow during severe relapse. Note complete absence of fat. × 500.

Fig. 2. Case 1. Same as Fig. 1, but under higher power to illustrate extensive hyperplasia of megaloblasts, with mitoses, and relative scarcity of normoblasts. × 1000.

Plate 58

Fig. 3. Case 1. May 19, 1925. Drawings of same material as Figs. 1 and 2. To illustrate hyperplasia of megaloblasts and numerous mitoses of megaloblasts. × 1250 (approx.).

Fig. 4. Case 1. Drawing similar to Fig. 3. × 1250 (approx.).

Plate 59

Fig. 5. Case 1. March 12, 1926. General character of marrow during a remission. Note large deposits of fat and small islands of myeloid cells between the fat globules. × 100.

Fig. 6. Case 2. April 29, 1926. General character of marrow taken early in a remission. × 200.

Fig. 7. Case 1. March 12, 1926. Marrow during remission. Island of cells between fat globules to show predominance of normoblasts. Very few megaloblasts. × 750.

Plate 60

Fig. 8. Case 2. March 12, 1926. General character of marrow during relapse. Hyperplasia of megaloblasts and displacement of fat. × 500.

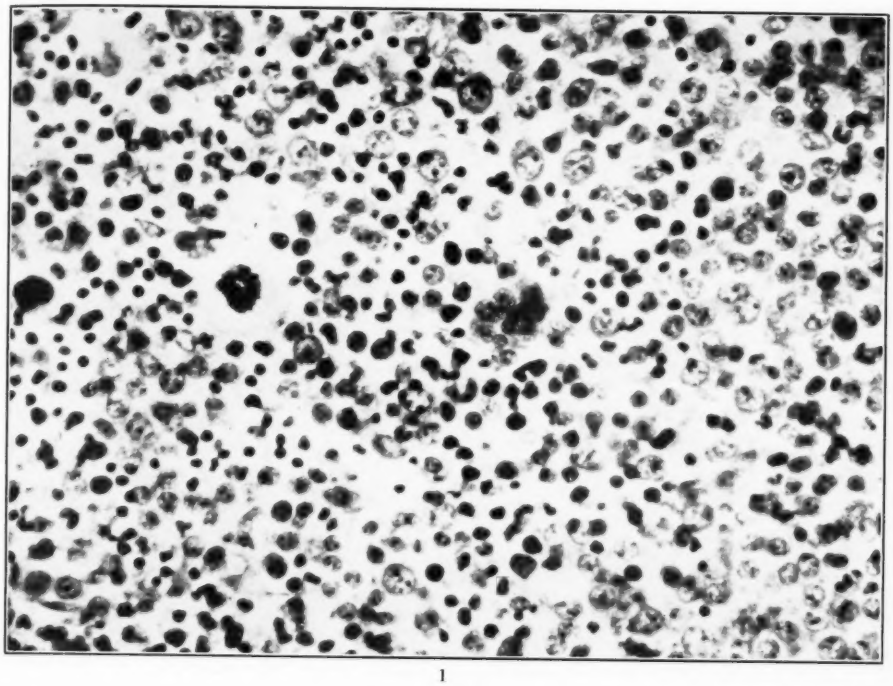
Fig. 9. Case 2. April 29, 1926. Masses of normoblasts and erythrocytes in the intersinusoidal capillaries between fat globules during a remission. Very few megaloblasts present. × 1500.

PLATE 61

FIG. 10. Case 3. Jan. 18, 1926. To illustrate the extensive hyperplasia of megaloblasts and the presence of fat in the terminal relapse. × 500.

FIG. 11. Case 3. Sept. 2, 1925. General character of marrow as patient was going into a relapse. Note presence of fat containing fat cells, and also marked hyperplasia of megaloblasts. × 750.

FIG. 12. Case 5. Sept. 2, 1925. Clump of megaloblasts developing in intersinusoidal capillary between fat cells. Note mitosis. × 1000.



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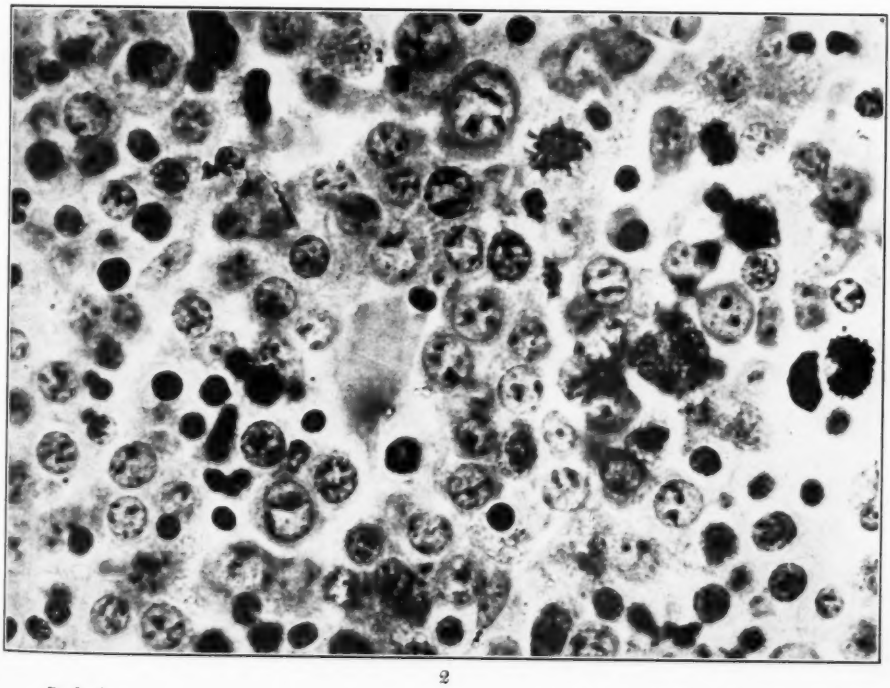

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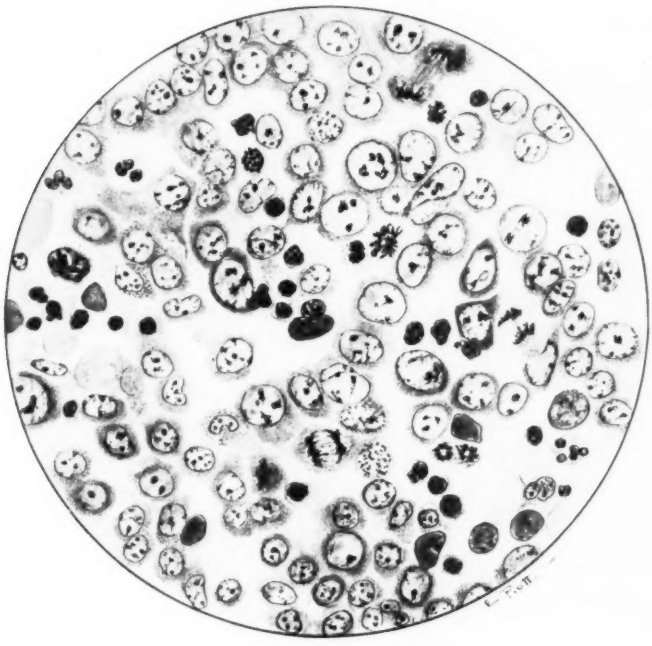

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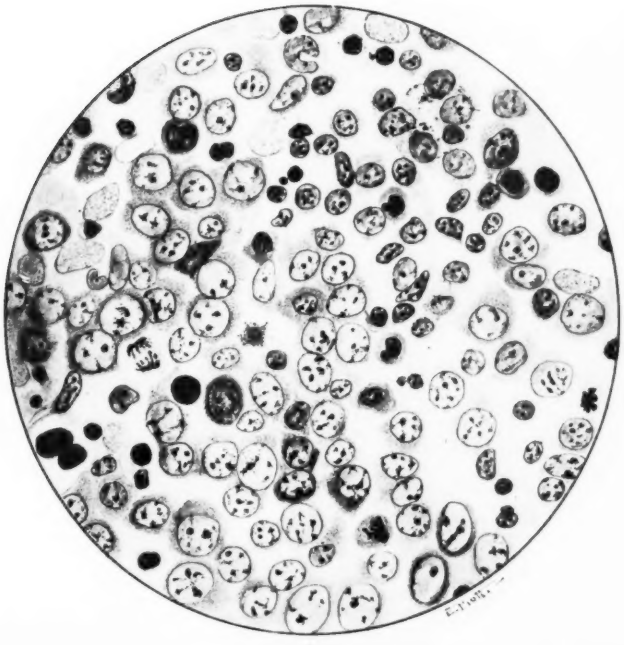
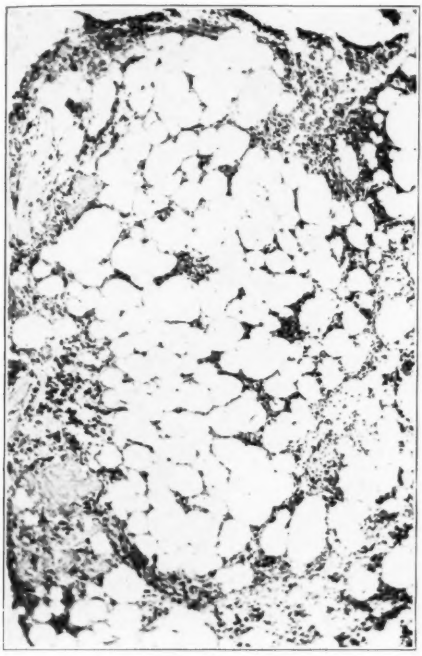


4

Peabody Bone Marrow in Pernicious Anemia



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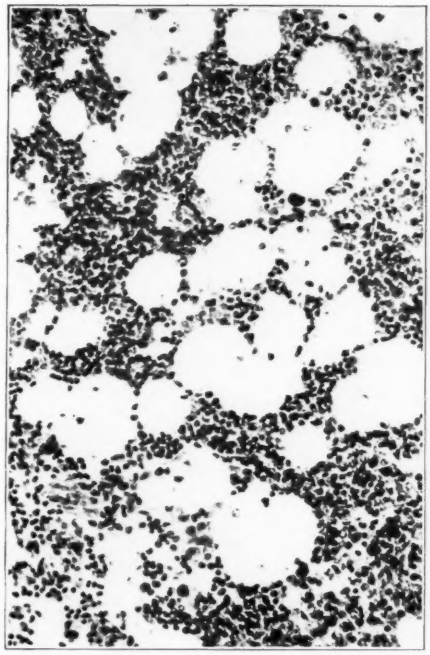

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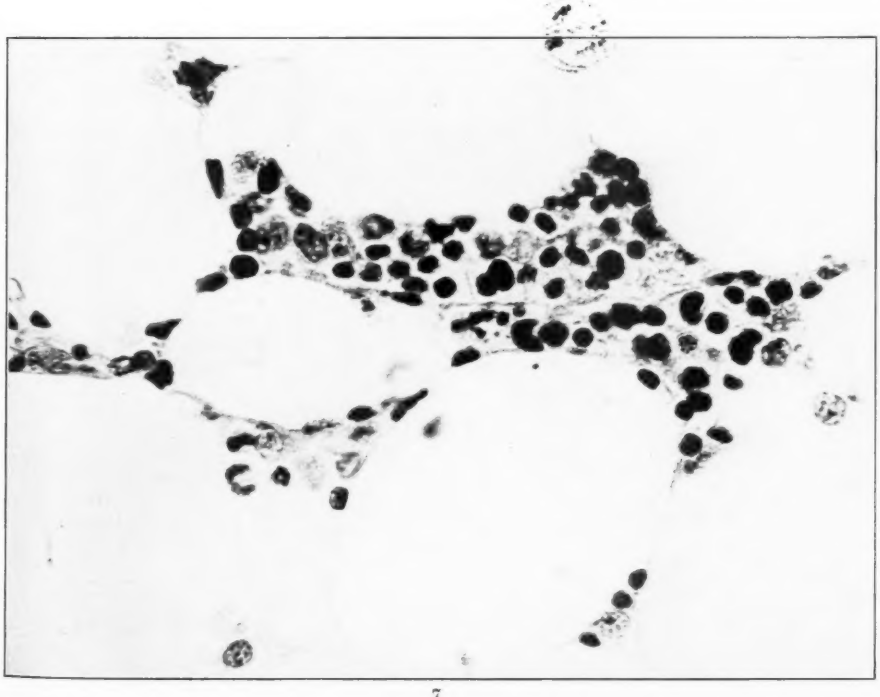

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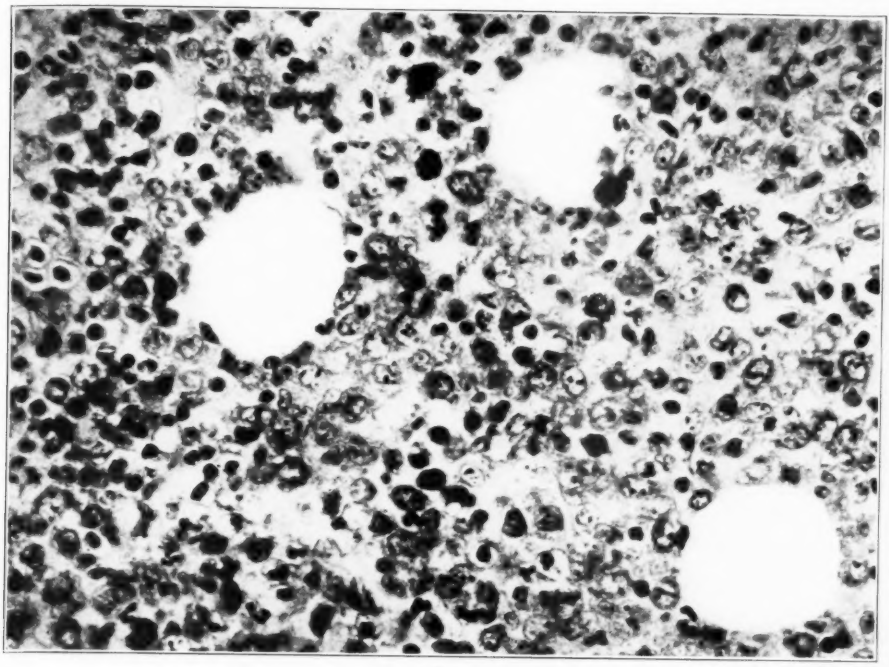
Peabody Bone Marrow in Pernicious Anemia



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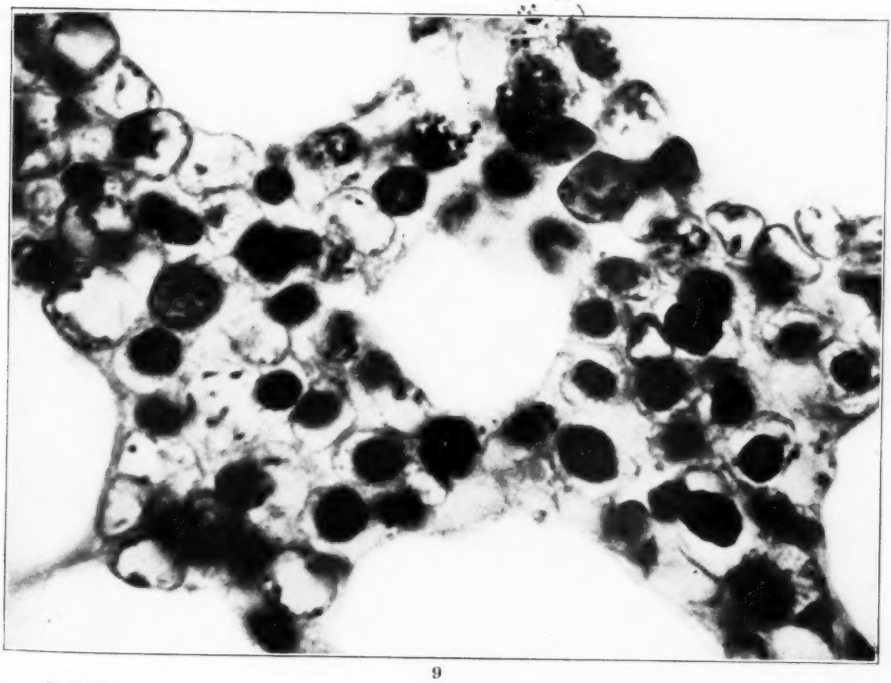

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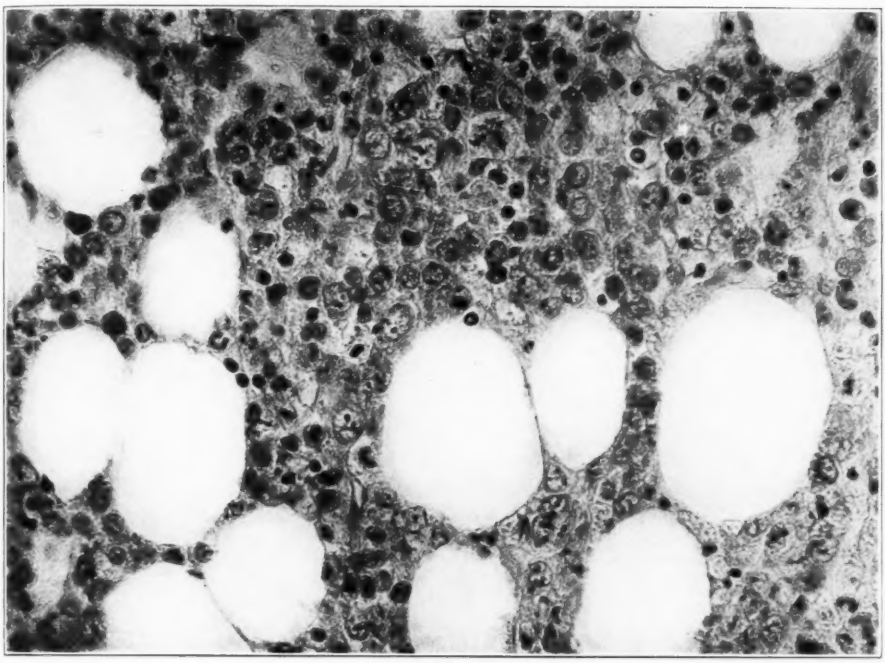
Peabody

Bone Marrow in Pernicious Anemia



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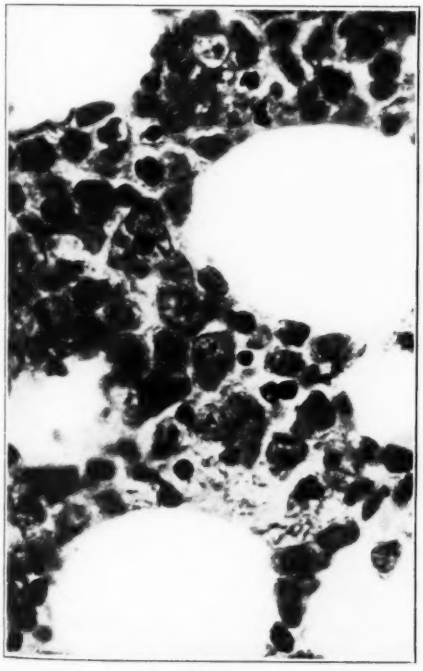

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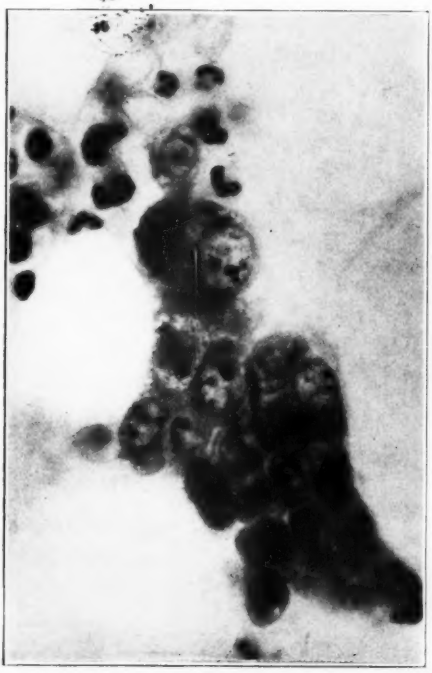

12

Peabody

Bone Marrow in Pernicious Anemia

THE TRANSFORMATION OF A MALIGNANT PARAVERTEBRAL SYMPATHICOBLASTOMA INTO A BENIGN GANGLIONEUROMA*

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INTRODUCTION

Among the rarest of the tumors of the central nervous system are those composed of the highly specialized nervous elements themselves. There are some pathologists who even doubt the existence of growths in which these elements chiefly participate, though, as is well known, occasional fully developed nerve cells may be disclosed in some of the gliomas when examined by the more modern methods of differential staining.

Certain tumors which take their origin from the sympathetic nervous system and more particularly in association with adrenal rests were described by J. H. Wright [1] as neuroblastomas; and subsequently tumors with a similar cellular architecture found in the central nervous system came to be thus designated. Wolbach and Bailey,[2] for example, in a brief article on the subject employed this term, as others had done, for certain tumors, with stroma, composed of round cells having delicate cytoplasmic processes, many examples of which had come under observation in the clinic.

Bailey and Cushing,[3] however, in a comprehensive reinvestigation of the tumors of the central nervous system occurring in the Peter Bent Brigham Hospital series found exceedingly few examples of what they felt justified in calling neuroblastomas. They consequently were disposed to regard this term as previously employed for the J. H. Wright type of tumor, at least in so far as it occurs in the brain, as a misnomer. In their opinion, these tumors arise from primitive and undifferentiated elements ("medulloblasts") of the nervous system. Potentially the cells therefore may be capable of differentiating into either glial or nervous elements, but, as a matter of fact, their disposition is toward a transformation into glia. Accordingly, for these tumors which are frequently encountered in the midcere-

* Received for publication December 24, 1926.

bellum of children and which are a common source of the so-called "sarcomatosis of the meninges," they preferred the name medulloblastoma.

In surprisingly few of the 400 tumors comprised in their study were true nervous elements identified, and in only three did they feel that the term neuroblastoma was justified. Among the true tumors of the central nervous system in the collection at the time of their report, no example of the still more highly differentiated type of growth known as a ganglioneuroma had been observed.* However in their monograph brief reference was made (pp. 93–94) to the case that we propose to report here in some detail. Though intraspinal, it was an extradural lesion and consequently belongs more properly in the category of peripheral than of central nervous system tumors. Both from the standpoint of its clinical history as well as on pathologic grounds, the case has most unusual features. It was included among the eighty examples of sarcoma which reacted favorably to bacterial toxins, as reported in 1913 by Dr. William B. Coley at the 3rd International Conference of Cancer Research.†

Case Report

P. B. B. H. Surg. No. 14560. *A paravertebral swelling in the back of a paraplegic infant had proved on exploration (1911) to be a malignant tumor, "a spindle-celled sarcoma." Under Coley's toxin treatment the growth disappeared but the paraplegia persisted. A laminectomy 10 years later (1921) disclosed an intraspinal but extradural tumor which proved to be a "ganglioneuroma." Improvement of paraplegic symptoms during succeeding 5 years.*

May 18, 1921. Admission of William W., aged 11, with the complaint of spastic paraplegia. From the child's father, a physician, checked by Dr. Coley's published record of the case, the clinical history may be pieced together as follows.

The child was born on June 29, 1909. In February, 1911, the father was thrown from a wagon while holding the infant in his arms. It was not known at the time that the child had been in any way injured and the first suspicion of this was aroused a month later by a progressive weakness in the legs associated with a paravertebral swelling opposite the middle of the scapula on the right side. This swelling increased in size and the child was taken to be seen by a well known surgeon, Dr. Stuart McGuire of Richmond, Virginia.

The anamnesis records that the case was supposed to be one primarily of infantile paralysis to which the paravertebral swelling was unrelated. Gradu-

* A ganglioneuroma of the pineal body has since been encountered.

† The treatment of malignant inoperable tumors, with the mixed toxins of erysipelas and bacillus Prodigiosus. Case 38. Brussels, 1914.

Some of the inaccuracies in Dr. Coley's report, particularly in relation to dates and in regard to the situation of the lesion, which he states was above the clavicle, are herein corrected.

ally the weakness progressed up the trunk and by May 1, 1911, the arms could not be moved; and soon there was fever, nausea, vomiting and stupor. There was also loss of sphinteric control. The eyes became crossed and there was ptosis.

In view of these symptoms Dr. H. Allison Hodges, a neurologist, saw the child in consultation with Dr. McGuire and the opinion was then expressed that the syndrome was due to some intradural extension of a disease which was possibly tuberculosis, though a lumbar puncture was negative and there was neither stiffness of the neck nor Kernig's sign. Since the paravertebral swelling was increasing in size, it was decided at the father's request that an exploratory incision be made to determine the nature of this local lesion.

This preliminary story is important, if it can be relied upon, in showing that there was originally a widespread involvement of the central nervous system, which extended above the level of the swelling in the back. To continue:

The paravertebral tumor was by this time plainly palpable and semifluctuant. It suggested either a lipoma or a tuberculous abscess. Sometime in June, 1911, Dr. McGuire, by an incision parallel to the ribs (Fig. 1), exposed the growth and found a well defined tumor apparently springing from the lamina or right transverse process of the sixth thoracic vertebra. The lesion was thought to be inoperable, and after removing a piece of tissue for histologic examination, the wound was closed. This tissue was diagnosed fibrosarcoma and the prognosis seemed hopeless.

The surgical incision healed promptly but since the tumor continued to increase in size, treatment by toxins was begun (*circa* July 1, 1911) by the child's father under Dr. Coley's direction.

Apparently it was not until the next year, in March, 1912, that the case was first seen by Dr. Coley. At that time Dr. James Ewing, after an examination of the original slides, stated that the growth was unquestionably "a malignant tumor which might very well be called a sarcoma." But this he qualified by the further remark: "I am inclined to think it is either an endothelioma secondary to the cerebral growth, or possibly a neurocytoma derived from misplaced nerve tissue in the cranium."

This statement is quoted to show that the view still prevailed that there had actually been an intracranial process and that the local lesion in the back was not the sole cause of the clinical picture. A detailed neurologic examination of the child made on March 19, 1912, by Dr. Foster Kennedy would appear to settle any doubts that might prevail on this score at the present day. He is quoted as follows:

On examination the child shows marked cerebellar attitude of the head, though the attitude referable to one lobe is not constant. Frequently there is a marked tremor of the head, precisely of the same character as that seen in advanced cases of disseminated sclerosis of the cerebellar or medullary type, or of advanced cases of Friedreich's ataxia. The pupils are brisk, the light and accommodation equal, central and regular in outline. There is no ptosis. There is marked nystagmus on lateral conjugate movement, either to the right or to the left. Query, weakness of the left sixth nerve. Jaw deviates always to the right, therefore, query, right motor fifth nerve affected. The masseter on the right side contracts less well than that on left. Child apparently hears on both sides. There is obviously no defective vision, no changes in the sensibility of the face, nor is there any facial palsy. The tongue comes out straight, and there is no tremor, or wasting. Palate normal.

Upper extremities. There is marked motor ataxia in both arms of definitely cerebellar type. (*N. B.* This ataxia is not in any way dependent on any sensory defect.) Diadokokinesia right and left. The arms are very strong for all movements. There is no wasting. No paralysis in the legs. All movements can be performed, but the ataxia manifested in the arms is present in the lower limbs also. The sensory condition is everywhere normal, that is to say, the child appreciates pin-prick, touch and temperature everywhere. *There is no segment of anesthesia or hypesthesia in any way corresponding to the situation of the tumor in the mid-dorsal region.*

Reflexes. Arm jerks normal. Abdominal reflexes right and left, present and equal. Knee jerks increased equally. Double extensor responses. Owing to inattention of the child combined with gross nystagmoid movement of the eyeballs, it was difficult to get a clear view of the optic discs. The veins in each ocular fundus were very large. Outer edges of discs were seen in glimpses and were apparently clear, and did not present the sinuosity of outline nor the blurring usually seen after the subsidence of a neuritic process.

We are told that the child has improved very greatly in the past six months not only as regards weight and general nutrition, but in regard to power and ability to perform movements. It is obvious, however, that there is still a gross cerebellar lesion.

These notes made by a highly competent observer surely indicate that the cerebellar symptoms at the time were predominant and that the spinal paraplegia had largely disappeared. Did the story end here, one might well enough assume that the symptoms had been produced, as Dr. Ewing's qualifying remark suggested, by one of the common tumors (medulloblastomas) of the fourth ventricle which had inoculated the cerebrospinal spaces and caused a spinal implantation with paraplegia, which in certain rare cases is known to disappear spontaneously.

It may be pointed out, however, that the child was then under 3 years of age (2 years, 9 months, to be exact); also that the reflex movements of the lower extremities in transverse lesions of the cord were at the time imperfectly understood, and even today when

observed in infants may be difficult to distinguish from spontaneous movements. It would appear, nevertheless, that the intracranial and cerebellar symptoms were more pronounced at the time than were those referable to the spine.

The child's father continued the injection of the toxins, and eight months later (Nov. 23, 1912) wrote to Dr. Coley as follows: "There is no indication of the return of the growth on the back. He has never regained the use of his legs though he can move them better and they show no signs of wasting or contractions. His eye symptoms are also better. He is hearty and well developed, does not seem to suffer any, and is bright and full of life." And after another eight months (July 25, 1913): "Am glad to say that my little boy's condition is somewhat improved. He can use his legs but little; he can move them but has no strength in his knees. There is no sign of a return of the growth on his back, and his general health is good; his mind seems bright. I give him four minims of the toxins about every third day." During this time apparently the cerebellar symptoms, if such they were, seem to have fallen into the background of the picture.

For the ensuing eight years, the child, though remaining paraplegic, thrived and developed in all other respects. He had acquired an automatic control of the bladder and rectum and by 1919 had learned to balance himself awkwardly on crutches. As the spinal condition seemed stationary the father had finally come to the conclusion that an exploratory laminectomy should be undertaken. Hence the child was admitted to the Peter Bent Brigham Hospital under the date specified, just ten years after his original operation. The child was well nourished, healthy, coöperative, and had *no discomforts whatsoever*.

May 18, 1921. *Neurologic Examination.* This showed absolutely no signs of involvement of the brain or upper spinal cord. There was not a trace of the nystagmus, diplopia, ptosis and so on, described in the previous history. In the back was the scar of the old operation (Fig. 1). This was soft and movable, and palpation revealed no evidence of an underlying tumor. The X-rays of the spine, however, showed a cloudy area representing either dense fibrous tissue or ossification at the site of the original lesion. The outlines of the laminae, spines and transverse processes were clear and without evidence of having been involved in the disease.

The child was powerless to move the lower extremities but the slightest stimulus served to throw them into reflex movements which strongly suggested voluntary movements and even the patient was under the impression that he

had some voluntary control. He could stand alone with the support of crutches and by taking advantage of a sustained adductor spasm which held the knees together, the feet being separated and turned in. When this spasm relaxed he would fall unless supported.

Sensation was apparently completely lost up to the level of the sixth thoracic skin-field, but the sensory tests were difficult to interpret because a pin-prick or even a light touch would evoke spontaneous reflex movements which gave sensory impressions referred by the child to his legs.

The deep reflexes both at knee and ankle showed an easily elicited and sustained clonus. There was on both sides an active dorsal toe response to almost any form of stimulation, even such as the mere exposure of the legs by removing the bed covers. The cremasteric reflexes were active. Reflex erections were easily provoked by pricking the glans or picking up the skin of the groin. Under these circumstances the legs would flex and the bladder which could retain about 200 cc., would be emptied without sensation (Figs. 2 and 3).

Whatever may have been the condition ten years previously, certainly at this time there was nothing to be seen but the evidence of a total transverse spinal lesion at about the level of the sixth thoracic segment which corresponded to the site of the original paravertebral tumor. Moreover, the X-ray plates disclosed a somewhat dense shadow in the region of the former tumor which however did not affect the normal outlines of the adjacent laminae or transverse processes. It would appear from the hospital history that no preoperative diagnosis was ventured. No lumbar puncture was performed. It would almost certainly have shown a complete block with xanthochromia.

May 21, 1921. *Operation* (Cushing). *Laminectomy with disclosure of sharply defined extradural mass of dense non-infiltrating and non-adherent tumor tissue. This mass encircled and constricted the meninges and cord and apparently communicated with the relic of the original lesion through an enlarged intervertebral foramen.*

With the position of the old cicatrix as an indication of the original tumor site, the spines and laminae of the three adjacent vertebrae were at first removed. There was no apparent lesion of the bones but, on scraping off the periosteum from the laminae of the right side, a dense scar-like tissue was encountered in the spinal muscles which was taken to be the residuum of the original lesion. On the removal of the spines and laminae, instead of the usual extradural cuffs of fatty tissue, the canal was found to be filled with the same kind of dense scar-like tissue. An incision, made into this firm tissue, was carried down to a considerable depth without disclosing dura. The tissue was quite vascular.

Realizing that the exposure was insufficient, the additional spines and laminae of the two preaxial vertebrae were then removed. This brought into view the upper margin of the lesion with normal-appearing dura headward to it. Similarly the laminectomy of an additional postaxial vertebra exposed the normal dura caudad to the lesion. It therefore extended over approximately five spinal segments.

The exposed growth was then tilted up and lifted by blunt dissection away from the dura to which it was not adherent. As the fairly rigid posterior shell of tumor was broken away from its lateral attachments, it was evident that it extended around to the anterior aspect of the canal on the right side where it became much thinned out.

A fragment of this intraspinal tissue was immediately examined (Wolbach) and was reported as a probable ganglioneuroma. With this suggestion of a possible seat of origin for the tumor in a posterior root ganglion, which might account for its hourglass shape by coincidental extension into the paravertebral muscles and spinal canal, the wound, before closure, was reinvestigated. The extravertebral mass which had been taken to be cicatricial tissue was found to lie mainly between the laminae of the fifth and sixth thoracic segments just opposite the thickest portion of the residuum of the intraspinal growth. The two masses appeared to communicate by a narrow neck through the region of an enlarged intervertebral foramen.* A fragment of the dense extravertebral tissue was then removed for comparison with that which had been removed from within the canal.

Following the removal of the tumor the greatly compressed dura filled out to its normal dimensions and resumed its pulsations. It was not opened. In the hope that the procedure would suffice to release the cord from its constriction and permit a return of function, if such a thing were possible after so many years of compression. The wound was closed as customary in successive layers.

Postoperative Report. The child made an excellent recovery from the operation. Healing was perfect (Fig. 1). The preoperative symptoms remained unchanged at the time of his discharge.

Subsequent Notes. The boy's father soon wrote that he had improved greatly and had much better sphincteric control. Again, in 1923, owing to a persistent adductor spasm, some tenotomies were performed which helped him greatly. Now (August, 1926), five years since the laminectomy, he writes to say that conditions have continued to improve; that "the boy rarely has to void at night, and has not wet his bed for over two years"; and that he does not have to void frequently by day and can hold his urine for about fifteen minutes even after there is inclination to void. He reports that the cutaneous sensation seems "to be good," and states that "he gets up, dresses himself, and walks around the room without a crutch though most of the time he uses one crutch; he hitches his pony himself and drives around anywhere he pleases."

The Histology of the Lesion (*Wolbach*)

Gross Description. The intraspinal specimen consists of two fragments of fibrous tissue, one about 4 cm. long, the other slightly less, and about 2 gm. in total weight. Both fragments contain on palpation small, sharp, discrete nodules 1 to 2 mm. in diameter. The

* These hourglass-shaped tumors are well known. They are usually, however, of the type of the Recklinghausen tumor (neurinoma of Verocay). For the most part they are histologically unmistakable and have no relation to the tumor under discussion other than that they may have their histogenetic origin likewise in the cells of the neural crest. *Cf.*, a recent article on the subject by M. Borchardt (*Klin. Wchnschr.*, 1926, v, 636).

second piece of tissue from the extravertebral region consists of translucent white fibrous tissue.

From the first frozen sections at the time of the operation, the impression was obtained that it was degenerated dorsal root ganglion surrounded by cicatricial tissue, but on sectioning the larger piece, the material was found to consist of fat tissue traversed by bands of fibrous and nerve tissue. There were nerve bundles running in all directions, and masses of nerve tissue containing ganglion cells, many of which were much degenerated though easily recognizable because they were surrounded by capsular cells. Before the close of the operation a small piece of muscle from the erector spinae group, opposite the level of the tumor, and a few additional pieces of gray fibrous tissue possibly fascia were excised and submitted for study.

Microscopic Report. Many blocks were made of the tissue received. They were stained with eosin-methylene blue, Van Gieson's stain, and phosphotungstic acid hematoxylin. The tissue unfortunately had all been fixed in Zenker's fluid so that impregnation by Cajal's method was precluded. The tumor has invaded fat and voluntary muscle and consists mainly of fibrous tissue with islands of large cells having one to several nuclei. The fibrous tissue cells are characterized by abundant, wavy, intercellular material which stains pale brown with phosphotungstic acid hematoxylin and pink with Van Gieson, and is identical in appearance and staining reactions with the intercellular material of peripheral neurofibromas and tumors of the acoustic nerve (Fig. 4). With the phosphotungstic acid stain, one can trace long, loose, deeply staining processes which appear to be axis-cylinder processes, and here and there in the fibrous tissue there is a suggestion of myelin sheaths.

The large cells have many different shapes and sizes. Many of them resemble mature ganglion cells which send off dentrite-like processes for long distances. Others unquestionably have axone processes (Fig. 5), which can be followed for long distances and show minute granulations arranged linearly. The presence of Nissl substance in these cells is strikingly demonstrated by the Bielschowsky-Plein method (Fig. 6). A few of these cells presenting all the appearances of ganglion cells are surrounded by capsular cells.

In addition to the large ganglion cells, there are cells scattered in groups which exhibit the same intense basic staining and suggest the possibility of being embryonic elements in the tissue (Fig. 7). There

are no mitotic figures and there is no suggestion of activity on the part of fibrous tissue.

In stating that the adjacent spinal muscles and fat have been invaded by the growth, I wish to indicate that both of these tissues have been incorporated in the rather compact fibrillary (neoplastic) tissue, and that groups of small and large ganglion cells are found in such regions. The ganglion cells for the most part resemble those of sensory ganglia, but occasionally there are large fusiform cells with two processes, one at each end. In addition, here and there in the tissue are circumscribed collections of lymphoid cells surrounding small capillaries; they resemble lymph follicle formation rather than an inflammatory reaction (Fig. 8).

The Original Tumor. Through the kindness of Dr. Coley, a section of the original tumor from which the diagnosis of sarcoma had been made was forwarded for our inspection. At first glance the resemblance of the tumor to a fibrosarcoma (Fig. 9) with very little intercellular substance might be conceded. However, a peculiarity is immediately noticed in that the tissue is partitioned by connective tissue bands having all the relationships of a stroma in an epithelial tumor. With the stain employed (hematoxylin and eosin), it is impossible to see cell outlines, and the impression of spindle-shaped cells is obtained wholly through the oval outlines of the nuclei. The nature of the tumor, however, is made apparent by the presence of an intercellular substance consisting of extremely delicate fibrils which are stained a faint bluish pink. These fibrils occur in bands of considerable width separating cell masses from the stroma, and as large bands joining widely separated groups of cells. The size and grouping of the cells, the connective tissue stroma and the presence of the delicate fibrils which resemble cytoplasmic processes of the tumor cells, agree perfectly with the characteristics of the so-called "sympathetic neuroblastoma." In the slide submitted there are no cells of a more mature type such as have been frequently described in neuroblastomas arising in the adrenal gland.

Discussion

The importance of this long record lies largely in the interpretation of the pathologic changes which occurred in a tumor during a ten-year interval. Briefly, we have for consideration an intervertebral tumor of the hourglass type which lay partly within and partly without the spinal canal, but presumably was always extradural. The extravertebral expansion of the growth was explored in 1911 and was regarded as a spindle cell sarcoma. Subsequent study of a slide sent to us by Dr. Coley showed a structure typical of the sympathicoblastomas. Presumably this slide is representative of the tumor as a whole as it existed in 1911. The lesion was carefully

studied at that time by a number of pathologists, and the presence of a more highly differentiated type of cell would not have failed to excite comment. The tumor disappeared or diminished (cicatrized) under the influence of Coley's toxins. Symptoms of spinal pressure persisted, and years later (1921) the intraspinal lesion was identified as a ganglioneuroma, the more compact extraspinal remnant of the tumor showing the same characteristics.

A very thorough examination of the tissue removed in 1921 reveals no trace of cells similar in structure to those which in 1911 apparently constituted the entire growth. Instead we find a neoplasm composed chiefly of two elements, one representing ganglion cells and the other representing the growth of capsular and sheath of Schwann cells, which is the usual combination recorded in ganglioneuromas of sympathetic origin. Therefore, one is forced to conclude that the cells of this tumor as a whole have responded to the influences or factors governing the normal differentiation of the nervous system.

The change in the structure of this tumor during the ten-year interval may be regarded as throwing light on the potentialities of the cells in the so-called "sympathetic neuroblastomas," and supports the idea expressed by Bailey and Cushing that these lesions represent a tumor of a more primitive type of cell (sympathicoblast) than the sympathetic neuroblast. Whether the early story of a widespread process which involved the intracranial chamber with the production of cerebellar symptoms has any bearing on this localized tumor must remain purely conjectural.

It would appear without question that the proliferative activity of the normal growth subsided coincidentally with the administration of the bacterial toxins. It is safe to presume, from the study of the original tumor and its comparison with the tumor after a ten-year interval, that the lesion was originally an actively growing sympathicoblastoma whose cells, coincident with loss of proliferative activity, came to be differentiated in time into ganglion cells and into sheath and capsular cells. Whether or not this is the correct interpretation of the process, the case, from the pathologic standpoint, is a unique one.

Literature

Comparatively few ganglioneuromas have been recorded. John Shaw Dunn [4] in 1915 collected fifty examples from the literature. The great majority of them (forty-one cases) were situated, as might be expected, in some part of the sympathetic nervous system. There were four cases in its cervical portion, five in the thoracic and twenty-nine in the abdominal chain or its branches, eleven having been in the region of the adrenal gland.

Their rarity among intracranial tumors is shown by the fact that out of more than 1000 histologically verified cases in the Peter Bent Brigham clinic, only one tumor that might be called a ganglioneuroma has been encountered and that arose from the pineal body. When they do occur in the intracranial chamber they usually take their origin from the cranial nerves. Those which actually arise from the central nervous tissues themselves are with difficulty distinguished from heterotopias. Achucarro [5] and Lhermitte [6] have each described such a tumor in the cerebellum, Pick and Bielschowsky [7] one in the medulla oblongata, Schmincke [8] and Dumas [9] in the cerebrum, and both Greenfield [10] and Robertson [11] have encountered an example in the region of the tuber cinereum, probably arising from misplaced cells of the ganglionic crest. Bielschowsky [12] has more recently described a case in which there were a number of small tumors in the ventricular walls which did not break through the subependymal glia; the author describes them as multiple ependymal ganglioneuromas.

As is well known, the sympathicoblastomas have often been described as consisting of two types of tissue. Masses of cells with scant cytoplasm and richly chromatinized nuclei, often arranged in rosette forms, are separated by connective tissue-like bands which with specific stains give the reaction neither of collagen nor of glia. They are for the most part highly malignant lesions and produce metastases, the undifferentiated cellular elements being those which metastasize.

Certain examples from the literature may be cited in evidence of the fact that these tumors originate from cells of a more primitive type than the neuroblast, and show a tendency in places to differentiate into fully formed nerve cells as well as into capsular or neurilemma cells. K. Martius [13] (1913) reported the case of a child 2½

years old who had been operated upon for a supposed malignant tumor of the thyroid and who promptly succumbed. At necropsy it was found that the growth had arisen at the angle between the first and second thoracic vertebrae and the adjacent rib. The growth was a typical sympathicoblastoma with neurofibromatous areas. In a separate nodule of the tumor which had a perfectly benign appearance fully developed ganglion cells were present.

A similar case was reported in the same year by Freund.[14] The patient was a child 4½ years old. The tumor was on the right side of the neck apparently at the level of the thyroid, and lay between the carotid artery and internal jugular vein. It was removed surgically. The tenth nerve was not seen at the operation. Histologically there were well differentiated ganglion cells as well as groups of less differentiated cells resembling those of sympathicoblastomas, while the bulk of the tumor had essentially the structure of a "neurofibroma," accompanied by axis-cylinder processes and some myelin sheath formation.

Another case in a somewhat unusual situation was described in Dunn's second paper.[15] The tumor which was surgically removed took its origin from deep in the sphenomaxillary fossa. The tissue proved to be moderately rich in the embryonic elements, which have wholly disappeared in our own case, but ganglion cells, sheath cells and axis-cylinders were typically represented. This, as Dunn states, represents an unfavorable omen as regards prognosis owing to the high potentiality for proliferation and metastasis possessed by these particular elements.

The unique feature of our present case report is the fact that an interval of a decade passed between the two occasions when the lesion was histologically examined. In all other recorded examples the tissue has been subjected to study at only one stage of development, or at least with but a short interval between a surgical biopsy and a postmortem examination.

Summary

A paravertebral swelling at the sixth thoracic level occurred in the back of a child 2 years of age, following a trauma. The lesion proved on exploration to be a cellular sympathicoblastoma (sympathetic neuroblastoma) which was mistaken at the time for a sarcoma. The

tumor had apparently taken its origin from the region of an intervertebral foramen, and had extended into the spinal canal as well as into the spinal muscles. Apparently under the influence of Coley's toxins the activity of the growth subsided. Ten years later, owing to the persistence of a paraplegia, an exploratory laminectomy was performed. This disclosed a relic of the former growth whose cells had become completely differentiated into ganglion, capsular and neurilemma cells.

Because of the unusual circumstances which permitted a study of the lesion at two remote periods, the case illustrates particularly well what has been pointed out by others, that a sympathetic neuroblastoma may be the precursor of a ganglioneuroma.

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DESCRIPTION OF PLATES

Plate 62

Fig. 1. Showing the oblique white scar of the original operation (1911) and the recent scar of the laminectomy (1921).

Figs. 2 and 3. Showing (above) patient in May 1921 with spastic rigidity of the lower extremities; (below) effect of pinching a fold of skin in the groin. Note involuntary flexor contraction with elevation of right heel from table; also slight erection with automatic evacuation of bladder, the typical lower spinal mass reflex.

Plate 63

Fig. 4. From a portion of the tumor having the general structure of a "neurofibroma" and containing a bundle of incompletely myelinated nerve fibers. Phosphotungstic acid hematoxylin stain. × 500.

Fig. 5. From a portion of the tumor containing ganglion cells in some instances accompanied by capsular cells. Phosphotungstic acid hematoxylin stain. × 500.

Plate 64

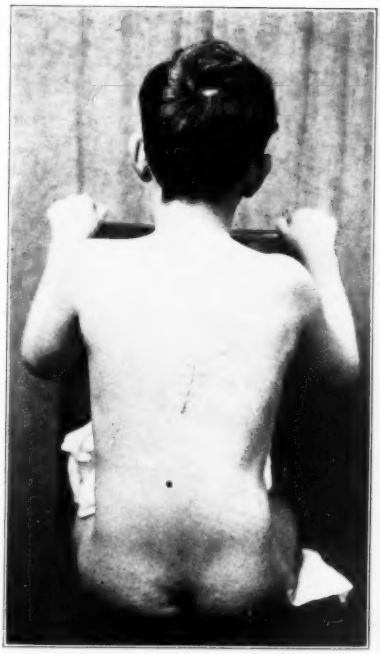
Fig. 6. Cells selected to show Nissl bodies. Bielschowsky-Plein stain. × 850.

Fig. 7. From a portion of the tumor containing nests of ganglion cells in various stages of differentiation, surrounded by fibrous tissue such as is found in "neurofibromata," and which represents tumor elements of neurilemma or sheath cell origin. The small cells with round nuclei and deeply stained cytoplasm are immature ganglion cells and probably represent multiplying elements. Phosphotungstic acid hematoxylin stain. × 500.

Plate 65

Fig. 8. Section to show collections of lymphoid cells which might easily be mistaken for persisting embryonal elements of the tumor. Perdrau's method. × 300.

Fig. 9. Drawing made from a section of the tumor removed in 1911. A field showing the nondescript appearance of the cells and the masses of delicate fibrils characteristic of a sympathicoblastoma. Hematoxylin and eosin stain. × 500.



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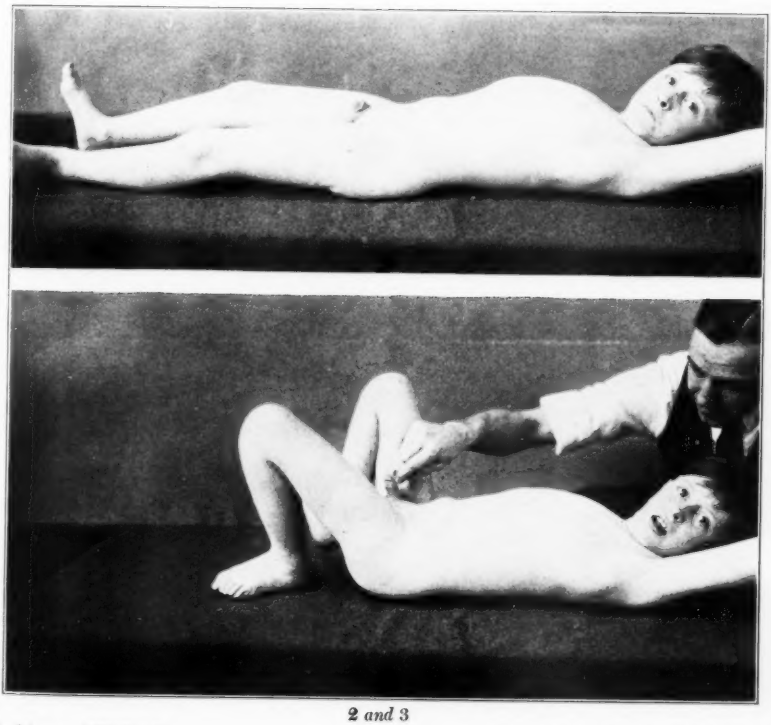
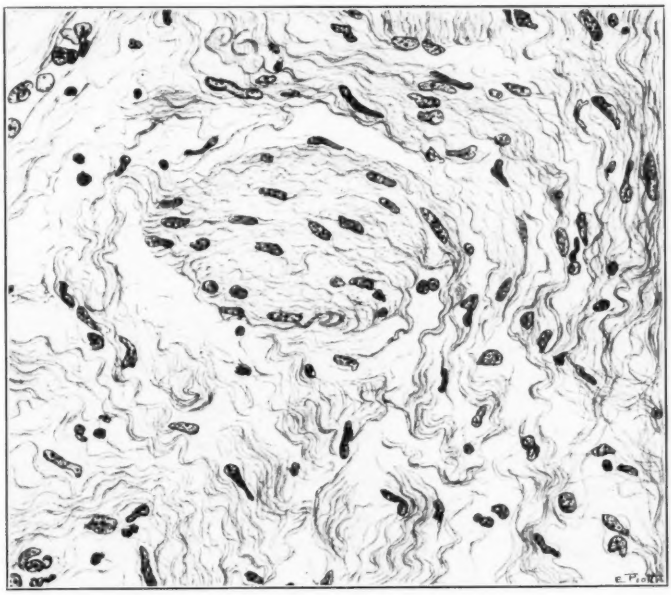


2 and 3

Cushing and Wolbach Paravertebral Sympathicoblastoma



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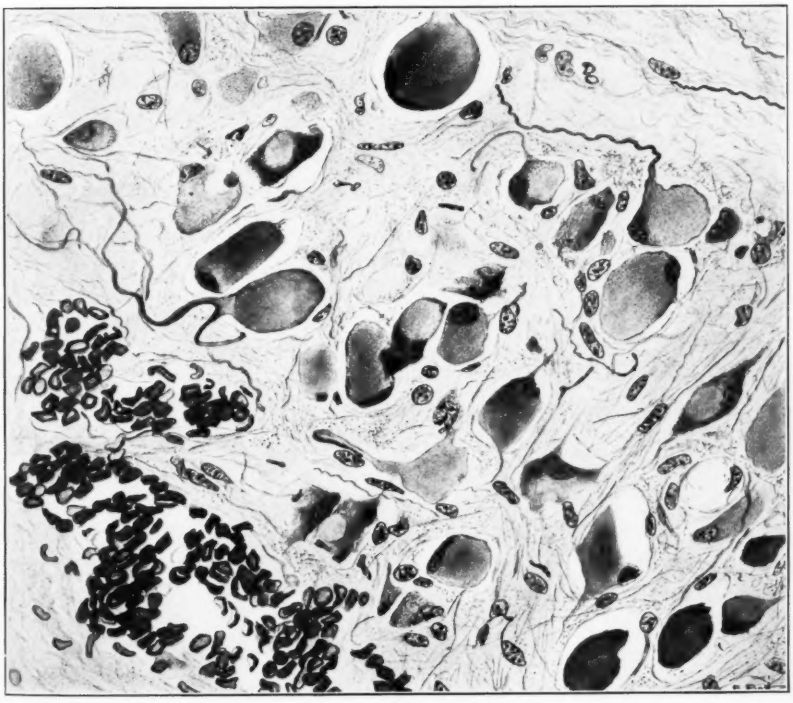

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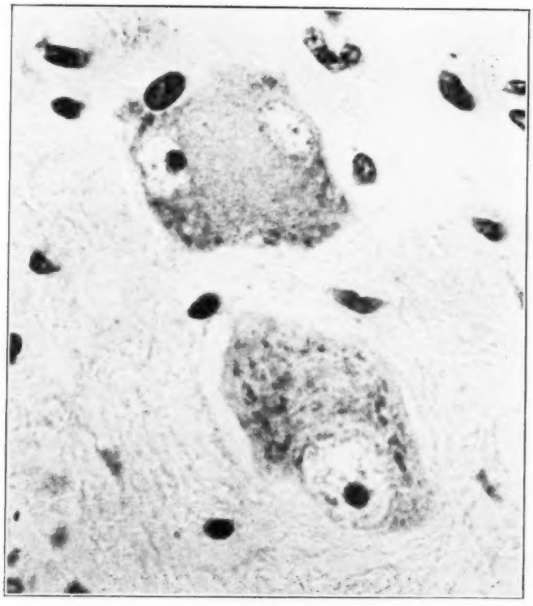
Cushing and Wolbach Paravertebral Sympathicoblastoma



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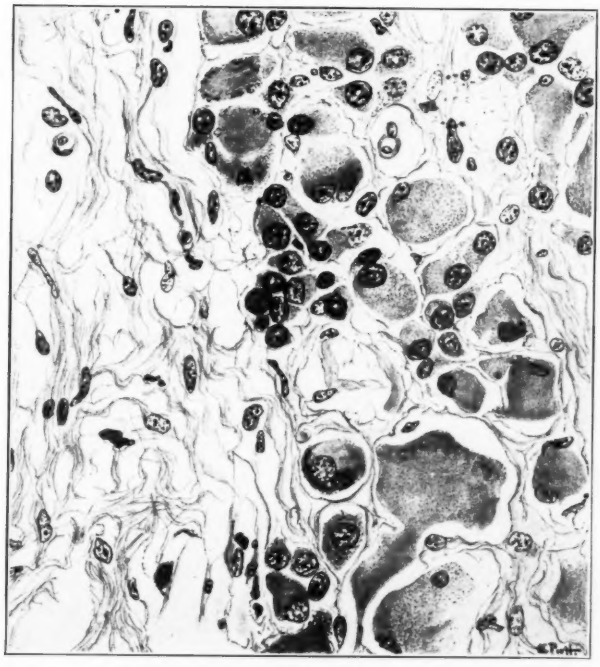

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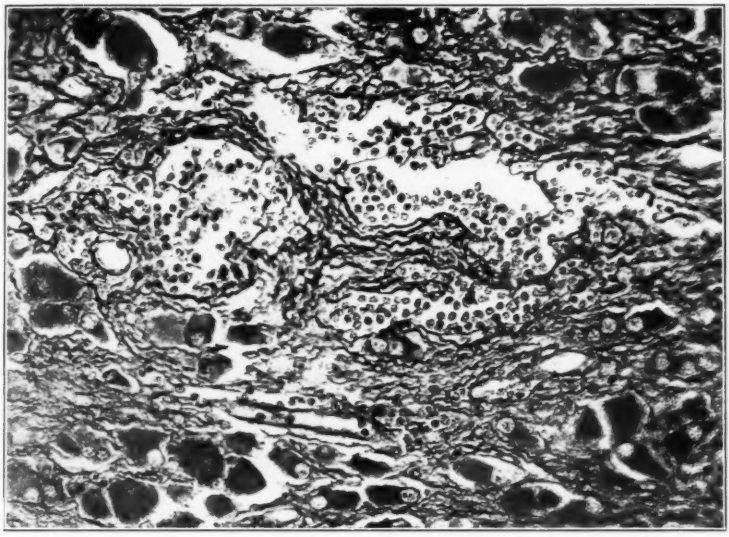
Cushing and Wolbach Paravertebral Sympathicoblastoma



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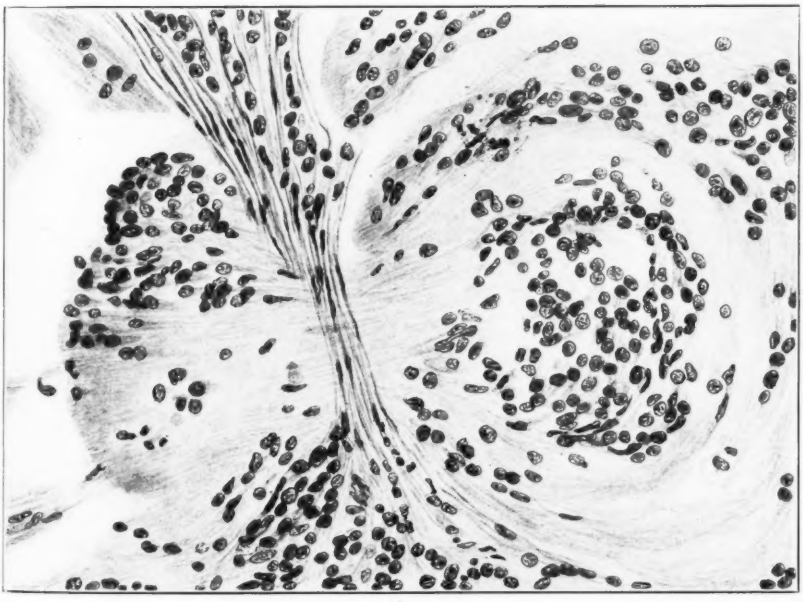

9

THE RETICULUM OF THE LUNG*

IV. ITS PRESENCE IN THE REPARATIVE PROCESS OF THE TUBERCULOUS LESION WITH AND WITHOUT CASEATION

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The occurrence of reticulum as a distinct type of connective tissue and its presence in normal tissues was first demonstrated by Mall.[3] By means of digestion experiments and chemical reactions he was able to differentiate it completely from elastic tissue, but found that it had many characteristics which showed that it was closely related to collagenous tissue. It will appear later that this has an important significance.

That reticulum is present in tuberculous lesions has been stated by numerous investigators; its demonstration, however, by special stains is due to the researches of Russakoff,[6] Miller,[5] and Foot.[2] Unfortunately no illustrations accompany the paper by Russakoff,[6] but the papers of Miller [5] and of Foot [2] contain numerous illustrations.

In his brief account of reticulum in tuberculosis, Russakoff[6] pays more attention to the transformation of reticulum fibers into collagenous fibers, and their possible origin, than to the tuberculous process.

Miller, in his various contributions, has illustrated with photomicrographs the relation of reticulum to various tuberculous lesions and, like Russakoff, found that it is a precollagenous type of tissue. He also found that the fibrils of reticulum are intercellular in their position, that they are not continuous with any cells but are associated with large mononuclear leucocytes which, as shown by Foot,[2] have been described by some twenty different names.

Foot [2] studied the formation of reticulum in experimental tuberculosis and, like Russakoff and Miller, found it to be a precollagenous tissue which appears to be "a product of preëxisting reticulum, as it is always continuous with it."

It is the purpose of the present communication to show that reticulum plays an important part in the reparative process of tuberculous lesions even after caseation has taken place.

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Since none of the human material at my command showed the early stages in the development of a tubercle it was necessary to make use of experimental material. Accordingly a series of rabbits were injected through the marginal vein of the ear with 0.1 mg. of a bovine type of tubercle bacillus. The animals were killed 7, 14, 28 and 56 days after the inoculation. The lungs were fixed in 10 per cent formalin and impregnated by Ferguson's [1] modification of Bielschowsky's silver method.

No distinct tubercles were found in the seven-day rabbit but small collections of mononuclear leucocytes were present. The fourteen-day rabbit showed well marked mononuclear or "epithelioid" tubercles in an early stage. These contained in their center a fine network of reticulum (Fig. 1). The fibrils which compose this network are coarser around the periphery of the tubercle where they join the reticulum in the remains of the alveolar walls. The fibrils are situated between the cells and nowhere do they show any connection with the cells.

At twenty-eight days the tubercles have increased in size (Fig. 2). The outline of the alveoli involved can be followed. The fibrils which make up the network of reticulum are coarser than in the preceding stage, especially around the periphery of the tubercle; only a few of the latter appear in the photomicrograph. The cellular contents of the tubercle consists of mononuclear leucocytes and a few scattered lymphocytes. A few of the mononuclear leucocytes show slight necrosis, but there is no evidence of beginning caseation.

At fifty-six days the amount of reticulum has very materially increased (Fig. 3). The cellular contents of the tubercle is the same as in the twenty-eight day tubercle. Only the central portion of the tubercle appears in the photomicrograph. The tubercle as a whole is surrounded by bundles of coarse fibers which begin to show here and there the characteristic reaction of collagen to the silver impregnation. No caseation is present.

The next stage in the reparative process was found in the human lung, and all the succeeding stages are taken from the same source. The tubercle occurred in the lung of a negro child 9 weeks old who had been under observation for six weeks. The photomicrograph is taken from the center of the tubercle (Fig. 4). The tubercle is made up of mononuclear leucocytes and lymphocytes. The lymphocytes are situated around the periphery of the tubercle, while the mono-

nuclear leucocytes are streaming towards the center. There is slight necrosis of a few of the mononuclear leucocytes near the center of the tubercle. The reticulum has become coarser than in the preceding figures, and around the periphery of the tubercle it has been converted into collagenous tissue. The reticulum is much more abundant than the figure shows, since only one plane can be photographed.

The final stage in the series showing the part that reticulum plays in the reparative process of tuberculous lesions in which caseation has not taken place, is from the lung of a white child, 10 years old, who died of tuberculous meningitis (Fig. 5). The network of reticulum, which forms the center of the tubercle, is much more compact than in any of the preceding stages. The coarse bundles of fibers seen in the upper part of the photomicrograph are collagenous, while in the lower part they show a transitional stage from true reticulum to pure collagenous fibers. The cellular contents of the tubercle is almost exclusively mononuclear leucocytes with a few scattered lymphocytes.

In the tubercles thus far described the reticulum has consistently increased in quantity and quality from a network of fine fibrils (Fig. 1) to a dense, compact network of coarse fibers (Fig. 5) which are gradually being converted into collagenous fibers. The ultimate ending of this metamorphosis is a small scar composed of collagenous fibers.

In none of the tubercles has there been any evidence of caseation and none of them has contained a giant cell. Medlar[4] has shown, however, that giant cells are a late production and that they represent "small areas of caseation or of simple necrosis around or into which mononuclear leucocytes have wandered," and indicate that a reparative process is taking place. In those instances in which slight indications of necrosis of some of the mononuclear leucocytes appear, there is an absence of polymorphonuclear leucocytes which are always associated with caseation and, since in every instance the network of reticulum has not been destroyed, there is evidence that no serious damage has been done and that the reparative process has become well established.

The tubercle shown in Fig. 6 is from the same lung as Fig. 5. This is an example of a tubercle in which caseation had commenced, but had been arrested before liquefaction had started, and reparation was taking place. The caseous material is being digested and ab-

sorbed. Extending through the caseous mass there is a large amount of reticulum which is but faintly shown in the photomicrograph. The tubercle itself is surrounded by a well marked band of collagenous fibers.

The lung, of which a small portion is shown in Fig. 7, was removed from a man 56 years old. He had been under observation for four years. The X-ray picture and the physical findings remained unchanged. It presents interesting features in connection with the reparative process in cases of pulmonary tuberculosis in which caseation has taken place.

A section through a portion of Fig. 7, stained with hematoxylin and eosin, is shown in Fig. 8. Under low power the tubercles (1 to 6) appear similar and take the rose-red tint of hyaline collagenous tissue but when impregnated with silver, a marked differentiation is brought out. In Fig. 8 the tubercles 1 and 6 appear to be confluent, but the silver impregnation shows that the slight depression above tubercle 1 marks out the boundary between the two tubercles. The five following photomicrographs are taken from the center of the tubercles and show transitional stages from pure reticulum to pure collagenous tissue.

There is so slight a difference between the tubercles marked 1 and 2 in Fig. 8 that they may be considered together. In each tubercle the center is made up of a network of reticulum (Fig. 9) which is rather more compact in tubercle 2 than it is in tubercle 1. Around the periphery of each tubercle there is a narrow band of collagenous fibers, while between the center and the periphery there are various gradations between fibrils of reticulum and collagenous fibers.

In tubercle 3 (Fig. 8), the center of the tubercle (Fig. 10) is made up of a network of coarse fibers of reticulum which almost immediately join still coarser fibers which show here and there the characteristic reaction of collagen to the silver impregnation. These latter fibers join the band of collagenous fibers which surrounds the tubercle. The band of collagen is more pronounced in this tubercle than in the others, due to the plane of section.

Fig. 11 is a section through tubercle 4 (Fig. 8), which gives the impression that it includes two alveoli. Possibly this is the case, for undoubtedly all the tubercles in the section are conglomerate. This appearance is the result, in this particular instance, of the photomicrograph including more of the left side of the tubercle than it does

of the right, in order that the increased number of collagenous fibers could be shown to better advantage. In the photograph, the center of the tubercle is that part which appears to be made up of a more open network than the remaining portion. This is due to the fact that in the central part there is still a remnant of the network of fine fibers of reticulum, while the remainder of the tubercle is made up of collagenous fibers. As can be readily seen, the majority of the fibers are cut obliquely.

Tubercle 5 (Fig. 8), appears to be made up entirely of interlacing bands of collagenous fibers (Fig. 12), but in a few places, notably in the circular area in the lower right quadrant, remnants of the network of reticulum can be seen. With the exception of these scattered areas, all the fibers contain collagen and take the characteristic yellow color imparted by the silver impregnation.

In Fig. 13 from the center of tubercle 6 (Fig. 8) no remnant of reticulum remains; the entire tubercle has been converted into collagenous tissue that joins the scar situated immediately above it. In time tubercle 1 would have been converted into collagenous tissue and the entire mass would have formed a compact scar.

In this last series caseation had already begun, but had been arrested (Fig. 6). Fibrils of reticulum had extended from the band of collagenous fibers which surrounded the tubercle into the caseous area, and mononuclear leucocytes were streaming into the caseous material. Some of the reticulum had become fragmented, due in all probability to the proteolytic enzyme of the polymorphonuclear leucocytes. These fragments, like the caseous material, would in time be absorbed. New fibrils of reticulum growing in from those present around the periphery of the caseation would replace them and, following the removal of the necrotic tissue, would fill the space occupied by the caseous material with a new growth of reticulum (Fig. 9). Both the reticulum already present and the newly formed fibrils become coarser, and the network they form becomes more compact as the reparative process progresses until finally the entire tubercle is converted into collagenous tissue (Fig. 13).

A comparison of the reparative process in the two series brings out some interesting results. In the first series, the reparative process was not complicated by necrosis, and the development of reticulum and its conversion into collagenous fibers was unimpeded; the result was a small, often insignificant, scar.

In the second series, caseation complicated the reparation of the tubercle. With the arrest of the caseation, and after the necrotic tissue had been digested and absorbed, a new growth of reticulum filled the space thus created, and from this time on, the reparative process proceeded as in the first series; the result was a scar but, of necessity, larger than in the first series.

The statement is not infrequently made that collagenous fibers grow from the periphery of a tubercle into its interior, the inference being that they are present at the periphery from the initial period. In a young tubercle there are, as shown in Fig. 1, no collagenous fibers around its periphery but only fibers of reticulum. From these fibers prolongations extend into the interior of the tubercle where they unite to form a network.

As the reparative process progresses in a tubercle, the fibers of reticulum about its periphery increase in thickness and gradually become collagenous. If the center of a tubercle is not invaded by polymorphonuclear leucocytes in sufficient numbers to cause caseation and complete destruction of the network of reticulum, this reparative process is constantly repeated until the entire tubercle is converted into collagenous tissue (Fig. 13).

The transition from reticulum into collagenous fibers can be followed in sections impregnated by the silver method in which the fibrils of reticulum are black, the collagenous fibers are a golden or a brownish yellow, while the thickened fibers of reticulum which have not acquired collagen are of a more or less gray tint. The factors which lead to the conversion of reticulum into collagenous fibers are, to me, unknown.

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DESCRIPTION OF PLATES

PLATE 66

FIG. 1. Rabbit's lung. Mononuclear tubercle fourteen days after intravenous inoculation. The outline of an alveolus is marked out by heavy fibers of reticulum. These fibers give origin to fine fibrils which extend throughout the tubercle and form a well marked network. The fibrils of reticulum are situated between the cells and have no connection with them. No polymorphonuclear leucocytes present. × 500.

FIG. 2. Rabbit's lung. Twenty-eight days after intravenous inoculation. The tubercle is larger and the fibrils of reticulum are coarser than in the fourteen-day tubercle. A few of the cells in the lower right quadrant show slight necrosis, but there is no evidence of caseation. × 500.

PLATE 67

FIG. 3. Rabbit's lung. Fifty-six days after intravenous inoculation. The increased amount of reticulum and the increase in size of the fibers is especially noticeable.

FIG. 4. From the lung of a negro child 9 weeks old. The dark bands around the periphery of the tubercle are made up of collagenous fibers. A few cells near the center of the tubercle show slight necrosis. × 500.

PLATE 68

FIG. 5. From the lung of a child 10 years old. The entire center of the tubercle is occupied with a network of coarse fibers of reticulum. The fibers in the upper part of the figure are collagenous; those in the lower portions show transitional stages between reticulum and collagenous fibers. × 500.

FIG. 6. From the same lung as Fig. 5. Caseation had taken place, but had been arrested. Fragmentation of the reticulum is shown. The entire caseation is permeated with fibrils of reticulum which do not appear in the photomicrograph. The tubercle is surrounded with collagenous fibers, some of which appear in the upper right quadrant. × 500.

PLATE 69

FIG. 7. Photograph of a portion of a lung which showed throughout its entire extent "healed" tubercles. × 1.5.

FIG. 8. Section from the lung shown in Fig. 7, stained with hematoxylin and eosin. The various tubercles numbered from 1 to 6, are shown in detail in Figs. 9 to 13. × 7.5.

FIG. 9. From the center of tubercle 1, Fig. 8. Shows a network of coarse fibers of reticulum. No collagenous fibers present. × 500.

PLATE 70

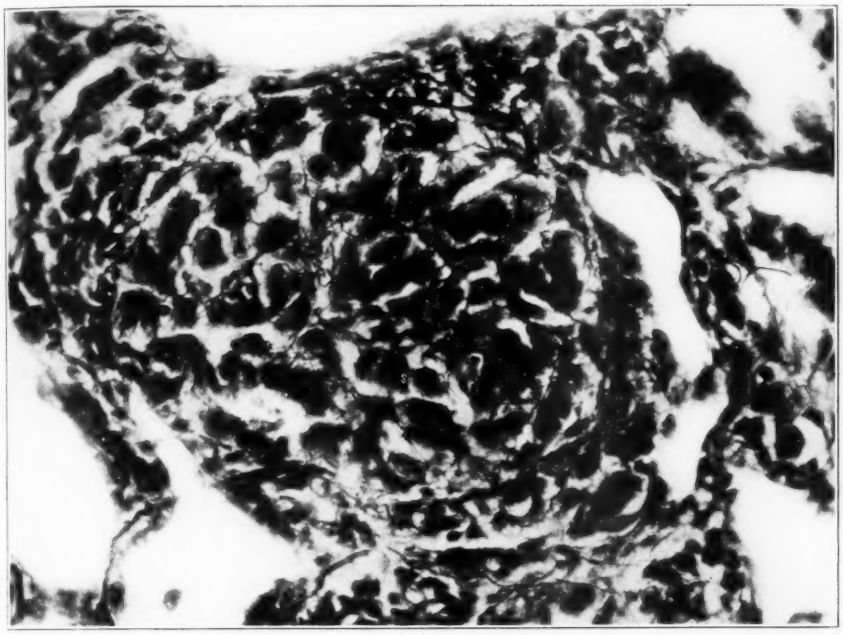
FIG. 10. From the center of tubercle 3, Fig. 8. The central portion is made up of coarse fibers of reticulum. The heavy fibers on either side are partly collagenous. × 500.

FIG. 11. From the center of tubercle 4, Fig. 8. With the exception of scattered fibers of reticulum in the lighter portion of the figure, the tubercle is made up of collagenous fibers. × 500.

PLATE 71

FIG. 12. From the center of tubercle 5, Fig. 8. This is almost exclusively made up of heavy bands of collagenous fibers. Small fragments of the network of reticulum which have not been wholly converted into collagenous fibers can be recognized.

FIG. 13. From the center of tubercle 6, Fig. 8. No reticulum is present. The entire tubercle has been converted into collagenous tissue.



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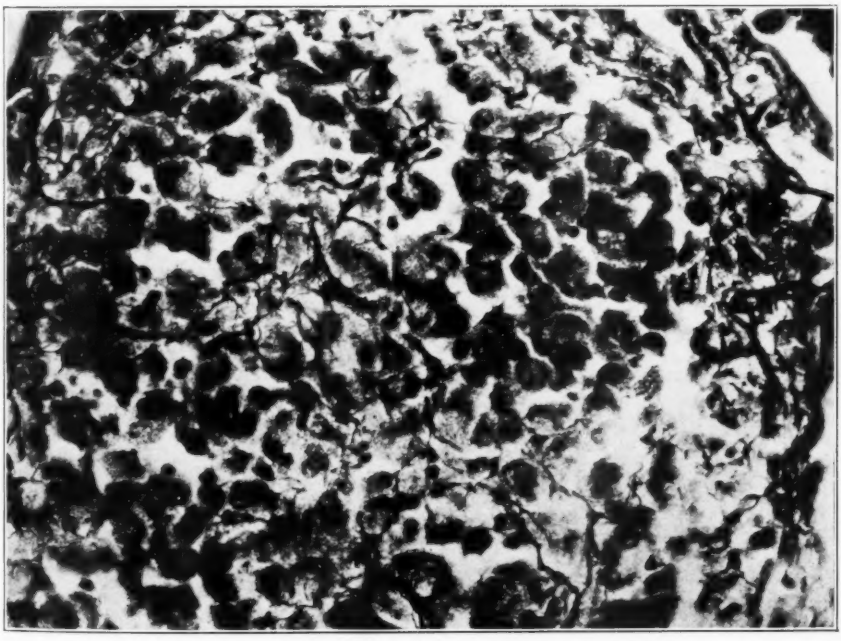

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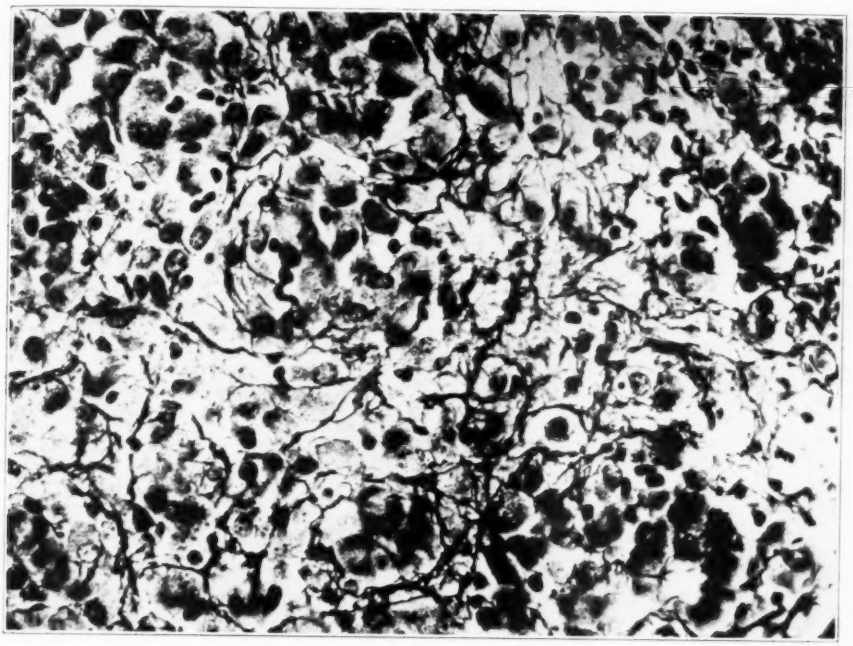
Miller Reticulum of the Lung



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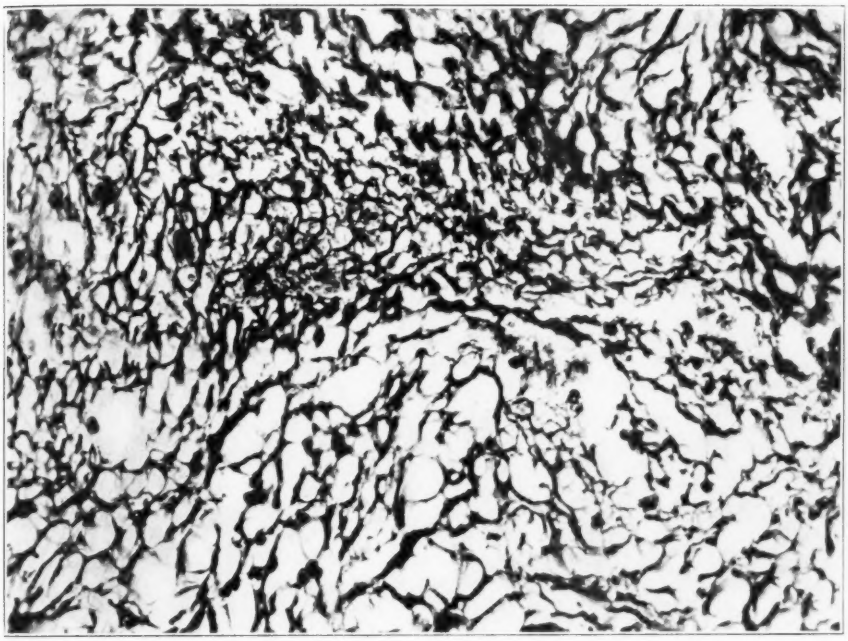
Miller Reticulum of the Lung



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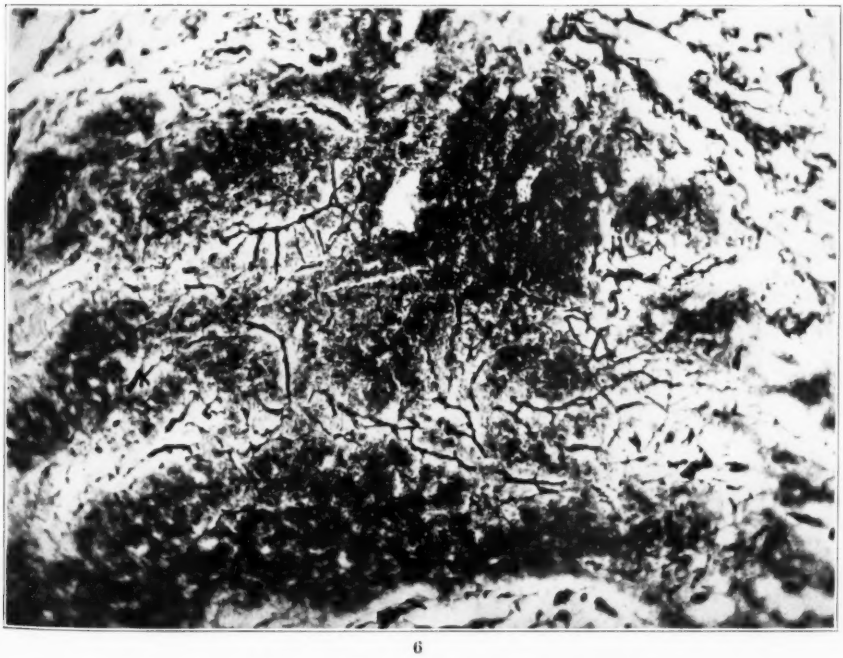

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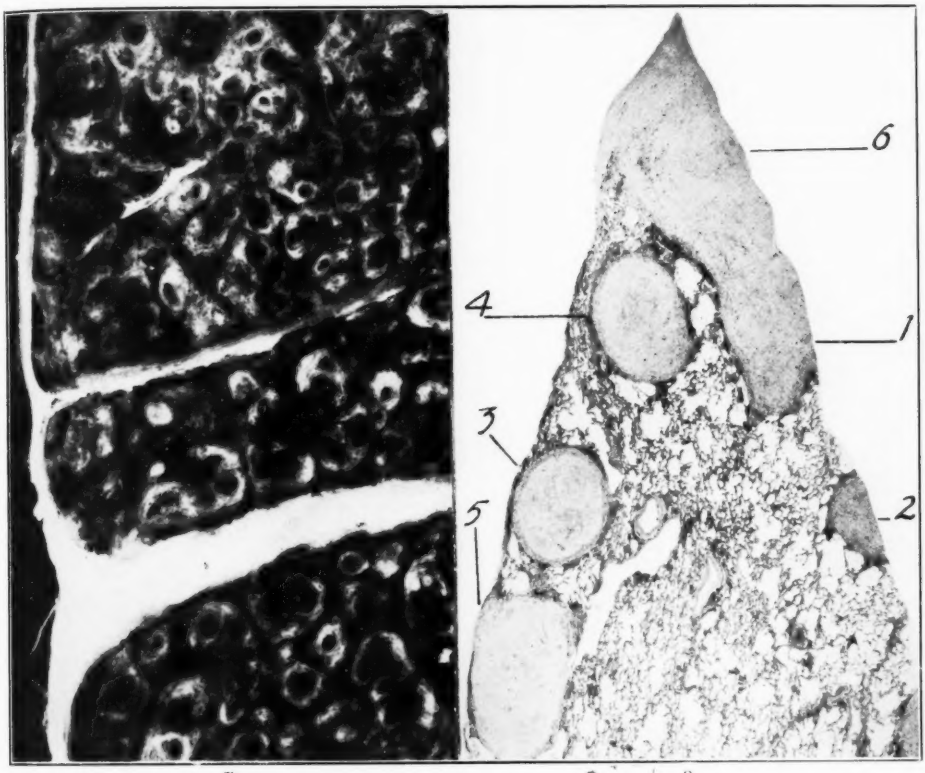

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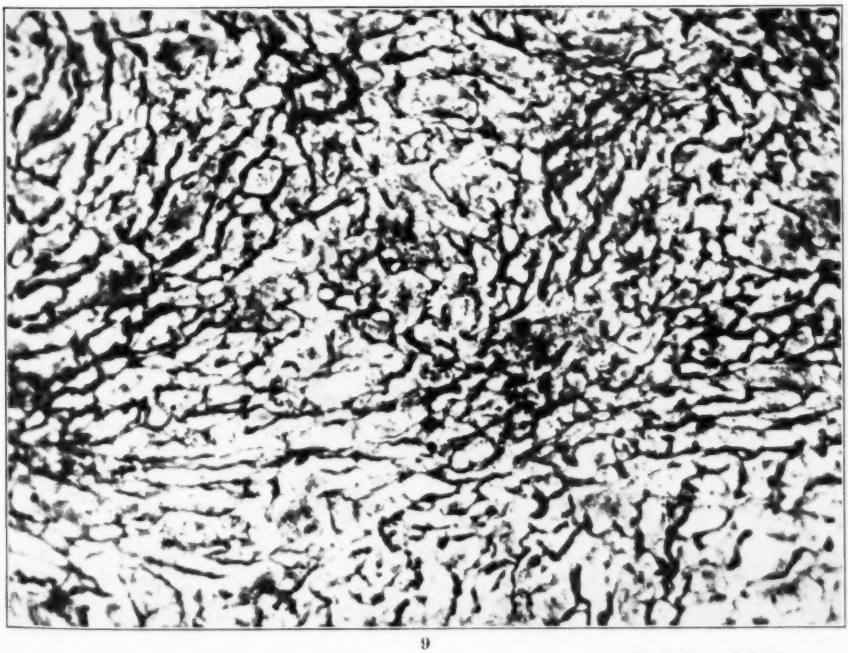

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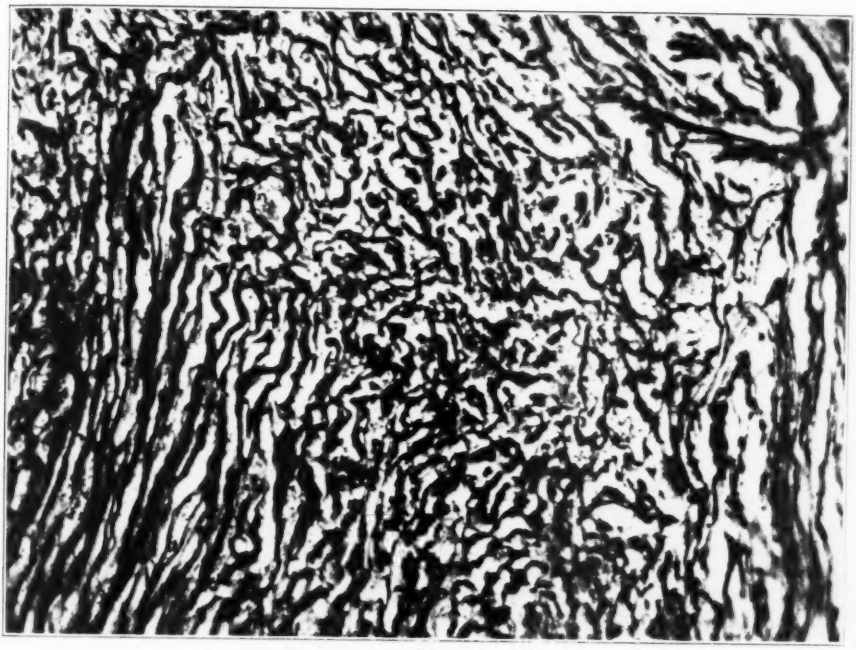

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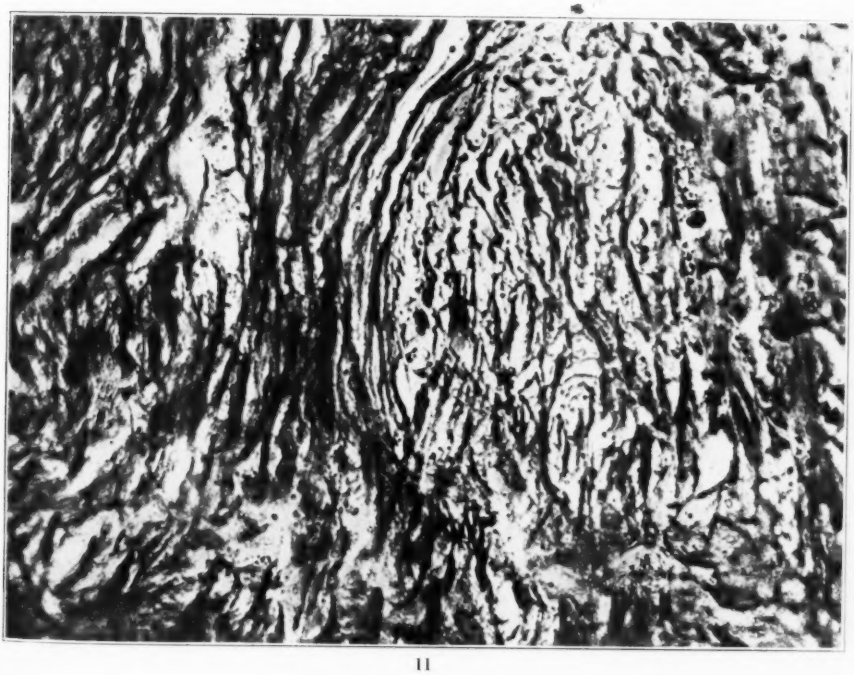

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Miller Reticulum of the Lung

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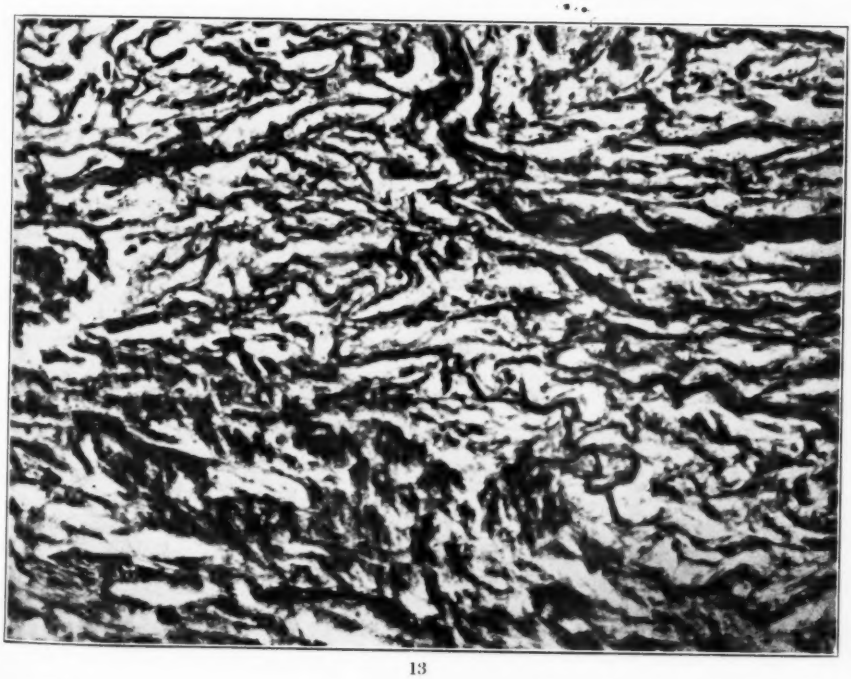

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Miller Reticulum of the Lung

PULMONARY SPIROCHETOSIS *

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In 1905 Castellani [1] described a form of hemorrhagic bronchitis with numerous pleomorphic spirochetes in the sputum. To the condition he gave the name bronchial spirochetosis. Since that time numerous cases have been reported from various parts of the world, including twenty-four from the United States. There has been considerable discussion concerning the specificity of the spirochetes in these cases. Castellani,[1] Fantham[9] and others described certain forms, varying in their morphology, of a species which they believe inhabit only the deeper air passages. Furthermore, certain characteristics are presented to differentiate these from the spirochetes found in the mouth and throat.

In a later report, Castellani [2] describes acute, subacute and chronic forms of the disease. Most of the subsequent writers have reported cases of the acute form, which is a mild disease and soon terminates spontaneously or responds quickly to the use of arsphenamine or tartar emetic.

These patients have cough, fever and the physical signs of bronchitis or broncho-alveolitis. The sputum is muco-purulent or blood-tinged and is laden with spirochetes. Castellani and others have found that the spirochetes in these cases vary in size and shape but may be separated into four types according to their morphology. In spite of this pleomorphism they believe that they may be differentiated from the spirochetes of the mouth by staining reaction, motility, etc. These authors have not mentioned the presence of fusiform bacilli. Fantham has described coccoid bodies which he thinks represent resting stages from which the spirochetes may develop.

All attempts to grow these organisms on artificial media have failed, and very little experimental work has been done. Chalmers

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and O'Farrell[3] selected a monkey that showed the presence of mouth and laryngeal spirochetes, then injected pneumococci into the throat, and chilled the animal. It developed pneumonia and died, but an examination of the bronchi and lungs failed to reveal an invasion of spirochetes from the throat. In a second experiment they injected the sputum from a case of bronchial spirochetosis into the trachea of a monkey. In thirty-six hours it became ill, developed a high temperature and a cough, and mucus from the throat was laden with spirochetes. After two days the symptoms subsided, the spirochetes disappeared and the animal recovered. Kline[6] has recently shown that gangrene may be produced experimentally by injecting spirochetes from a case of pulmonary gangrene into the traumatized tissues of guinea-pigs and rabbits, whereas gangrene is not produced in tissues that have not been subjected to trauma.

Several authors have found spirochetes similar to those described by Castellani, in the sputum and lung tissues of patients with pulmonary abscess or gangrene. It would seem, therefore, that these cases in varying stages comprise the chronic group described by Castellani. The diagnosis is often confused with tuberculosis due to the chronic cough, abundant foul-smelling sputum, emaciation, etc. The clinical picture of the acute form is not new as various authors would lead us to believe. Finding spirochetes in the sputum enhances our knowledge of the chronic cases only in so far as this secondary infection may mean a more prolonged and serious course with a greater tendency to gangrene. Foul-smelling sputum should always lead us to search for spirochetes. These organisms are usually overlooked in the routine examination of sputum, but are easily demonstrated when stained with a fresh solution of gentian violet, gentian violet followed with Gram's iodine, or better still, by the method of Warthin or Fontana.

Since we became interested in these spirochetes, we have studied the fresh sputum of at least a dozen patients with lung abscess or pulmonary gangrene, and along with the variegated bacterial flora present, we have always found a varying number of spirochetes and sometimes fusiform bacilli. In one case of lung abscess a small bronchial tree was coughed up and, when stained by the method of Levaditi, showed practically a pure culture of spirochetes. Sections of lung tissue from tuberculous and bronchiectatic cavities, where we expected to demonstrate spirochetes, have been studied but these

organisms were not found. In practically every case of pulmonary abscess or gangrene, however, which came to necropsy we have been able to demonstrate the organisms in abundance in the lung tissue.

The following illustrative cases have been studied during the past two years in the Pathological Department of the University of California Medical School.

Case Records

Case I. *Clinical Record.* A girl, aged 12, was admitted to the Pediatric Service of the University of California Hospital, with a draining chest wound following a rib resection for a post-operative pneumonia and empyema. Two years before admission she had her tonsils and adenoids removed, and shortly afterward, developed pneumonia of the left lung with empyema. One month later a rib resection was done and pus drained from the left pleural cavity. Ten months later, sections of four ribs were removed and the lung collapsed. The wound has drained since that time, and later, a physical examination showed a markedly emaciated, anemic, dyspneic child with a very foul breath and a foul discharge from the wound in the left chest. The entire left chest and right apex were dull to percussion. No breath sounds were heard over the left lung. The edge of the liver was felt well beneath the costal margin. The fever ranged about 102 F. The X-ray diagnosis was probable gangrene of the left lung. The child became apathetic, and died five days after admission to the hospital.

Pathologic Record. Left lung: weight 400 gm. The left pleural cavity was practically obliterated by thick, fibrous adhesions. Very large edematous lymph nodes were found at the hilum. The pulmonary artery was free from thrombi. The entire lung was firm and boggy with no suggestion of crepitation. Section showed multiple abscesses in the upper lobe, varying in size from 3 mm. up to 1.5 cm. in diameter, and filled with foul-smelling purulent material. A diffuse bronchiectasis was present. Between the abscesses there was a chronic interstitial pneumonia with an overgrowth of a dense, boggy fibrous tissue. Most of the lower lobe was occupied by a large ulcerating cavity divided into numerous compartments and containing a foul purulent material. The walls of this cavity showed an irregular, necrotic surface.

Right lung. Weight 240 gm. Recent fibrous adhesions had formed at the apices of the upper and lower lobes. The upper half of the upper lobe contained a large, thin-walled abscess which communicated with a similar abscess in the superior mediastinum. In the apex of the lower lobe there was another large abscess. All of these abscesses contained foul, purulent material like that found in the

left lung, but these were of recent formation and no interstitial pneumonia was present. A marked emphysema was evident in the portion of this lung which was not occupied by the abscesses.

No spirochetes had been found in the smears of the sputum or of the pus from the drainage wound in the chest, but after necropsy these smears were obtained and, after being restained, numerous spirochetes were found. Smears from the abscesses showed innumerable spirochetes. Sections from the walls of the abscesses were stained by the Levaditi method and showed various organisms in the necrotic exudate on the walls of the cavities, while in the deeper tissue and even invading the fibrous tissue, numerous spirochetes were found. In these areas no fusiform bacilli were seen in association with the spirochetes, but in the superficial areas some of the large organisms present might be suspected of belonging to this group.

Anatomic Diagnoses. Multiple abscesses, chronic interstitial pneumonia and bronchiectasis involving the entire left lung. Recent abscesses in the upper and lower lobes of the right lung and in the superior mediastinum. Chronic fibrous pleural adhesions obliterating the left pleural cavity. Recent fibrous adhesions at the apex and posterior surface of the right lung. Emphysema of the right lung. Thoracotomy with rib resection in the left axillary line. Marked lymphadenitis of the peribronchial nodes. Subacute splenic pulp reaction. Marked fatty infiltration of the liver. Moderate hydropericardium. Extreme emaciation.

CASE 2. *Clinical Record.* A man, aged 58, entered the Medical Service of the University of California Hospital, complaining of cough, fever and headache. He was subject to frequent colds, which he thought usually settled in the left chest causing pleurisy pains. A hacking cough had been present for about seven years which he supposed was due to excessive smoking. Three weeks before admission he had a head cold and then developed fever, a severe cough and a moderate amount of sputum. His cough and fever had persisted but his sputum had diminished in amount. A physical examination showed dulness at the base of the left lung posteriorly. The breath sounds were distant and bronchial in type. No râles were heard. The hemoglobin was 75 per cent, white blood cells 23,000, red blood cells, 3,800,000. X-ray examination of the chest showed a discrete mass in the lower portion of the left hilum 5 × 8 cm., and the base of the left lung diffusely gray with coarse mottlings. Bronchoscopy showed a nodular constricting mass at the bifurcation of the left bronchus. Throughout the course of five months in the hospital the patient ran a septic fever from 98.6 to 102.5 F. He finally developed a left-sided, slightly sanguinous pleural effusion of high specific gravity, and shortly before his death, began to expectorate a large amount of foul-smelling purulent sputum.

Pathologic Record and Anatomic Diagnoses. Primary carcinoma of the left bronchus with a large mass extending into the substance of the lung and into the lymph nodes at the hilum. Extensive metastases to the liver. Gangrenous bronchiectasis of the entire lower lobe of the left lung with ulceration of the surface, and a large encapsulated empyema between the lower lobe and the thickened pleura. Interstitial pneumonia, in the upper lobe of the left lung and in the all lobes of the right lung. Fibrino-purulent pleuritic exudate over the posterior surface of the right lung. Extensive fibrous pleural adhesions over both lungs. Marked hypertrophy and dilatation of the right ventricle.

The sputum and fluid obtained by thoracentesis had not been examined for spirochetes, but smears obtained from a gangrenous area in the lower lobe of the left lung showed numerous spirochetes. Sections stained by the Levaditi method also showed spirochetes in the gangrenous lung tissue.

CASE 3. *Clinical Record.* A young man, aged 18, was admitted to the University of California Hospital complaining of a persistent cough and much sputum. Two months before admission he had pneumonia with a right-sided pleurisy. He was in bed only a week and had apparently recovered, but four weeks later a sharp pain suddenly developed low in the right chest at the site of the previous pleurisy. A productive cough developed and he raised a large amount of greenish yellow, foul-smelling, purulent sputum. Physical examination showed a very foul breath, dulness to flatness at the base of the right lung and an area of hyper-resonance with amphoric breathing and bubbling râles. There was a positive Grocco's sign on the left side. The blood count showed 3,400,000 red cells and 12,700 white cells. The sputum was foul, yellowish green, and measured about 400 cc. in twenty-four hours. Smears showed pus cells, elastic tissue, streptococci and staphylococci, but no acid-fast organisms. No note was made regarding spirochetes. X-ray examination showed a shadow obliterating the right base and areas of rarefaction extending upwards and mesially. The whole lung above showed a mottled grayness especially extending from the hilum to the apex. Nine days after admission, portions of the eighth and ninth ribs were resected in the right axillary line. Dense pleural adhesions were encountered, and a small abscess cavity was found in the lower lobe. The patient died several hours after the operation.

Pathologic Record and Anatomic Diagnoses. Multiple abscesses of right lung with marked chronic interstitial pneumonia and bronchiectasis. Fibrous pleural adhesions obliterating right pleural cavity. Bronchopneumonia in the lower lobe of the left lung. Emphysema of the left lung. Recent thoracotomy with rib resection in right axillary line.

Right lung. There were several small abscesses towards the apex and larger gangrenous abscesses near the base. The intervening lung tissue showed a marked chronic interstitial pneumonia with fibrosis, and was diffusely mottled with yellowish dots indicating the presence of fat-laden phagocytic cells. The abscesses contained a greenish yellow, foul-smelling pus, and had tags of necrotic tissue on their walls. Smears from these abscesses showed numerous spirochetes and a few fusiform bacilli. Sections stained by the Levaditi method showed a large number of spirochetes in the lung tissue.

CASE 4. *Clinical Record.* A Chinese male, aged 39, entered the Medical Service of the San Francisco Hospital complaining of a bilateral chest pain and an irritating cough. Two months before entry he caught a cold, developed a fever and at times became delirious. One month ago he became worse, began to cough and raise considerable foul-smelling sputum which, at times, contained lumps of yellowish material. Hemoptysis occurred once. He recently developed bilateral chest pain. Physical examination showed marked emaciation, dulness at the bases of both lungs with flatness and other evidence of fluid at the right base. Moist râles were heard over both bases. The liver was enlarged and the fingers showed marked clubbing. The blood count was 3,088,000 erythrocytes, and 23,900 leucocytes. The fever was septic in type ranging from 100 to 104 F. The sputum was not examined for spirochetes but was negative for tubercle bacilli.

Pathologic Record and Anatomic Diagnoses. Large gangrenous abscess in the lower lobe of right lung communicating with an encapsulated empyema. Marked chronic interstitial pneumonia of right lung. Marked bronchiectasis of right lung, moderate in the left lung. Bronchopneumonia in both lobes of the left lung. Fibrous pleural adhesions obliterating the right pleural cavity. Few fibrous adhesions about the left lung.

Right lung. Weight 1270 gm. Thick, fibrous pleural adhesions covered the entire surface. Between the lower lobe and the thickened pleura there was an encapsulated empyema with foul-smelling greenish pus, which communicated with a large gangrenous abscess in the lower lobe. In the upper lobe there was thrombosis of a large vessel. Large bronchiectatic cavities occurred in all lobes, and there was marked interstitial pneumonia with no crepitant areas in the entire lung.

Left lung. Weight 780 gm. No abscesses were found, but there was slight bronchiectasis and bronchopneumonia in the lower lobe.

Smears from the abscesses in the right lung were stained by Warthin's method, and showed innumerable spirochetes and a few large

organisms that were probably fusiform bacilli (Fig. 3). Sections from the wall of these abscesses also showed numerous spirochetes in the tissue when stained by the Levaditi method.

CASE 5. *Clinical Record.* A man, aged 61, entered the San Francisco Hospital complaining of cough and aphonia. He had been coughing and raising large quantities of sputum for about three years. He never had hemoptysis. About two months before admission he caught cold during the night and completely lost his voice. The cough, with large amounts of sputum and a daily high fever, persisted and he became very weak. Physical examination showed marked dyspnea, teeth carious and advanced pyorrhea, and extreme emaciation. The left chest showed limitation of movement. There was dulness over both apices with many moist râles at the apices and bases. At the left apex signs of cavity formation were found. Examination of the heart was negative. Wassermann tests were repeatedly negative. There was a leucocytosis of 15,000 with 58 per cent polymorphonuclears. The X-ray examination suggested tuberculosis of the left lung, and upper lobe of the right lung. The patient had a septic temperature and raised large amounts of foul-smelling sputum during his three weeks in the hospital. Examination of the sputum failed to show tubercle bacilli, and no note was made regarding the presence of spirochetes. The day before his death he developed signs of acute pulmonary edema and failed to respond to treatment.

Pathologic Record and Anatomic Diagnoses. Multiple abscesses in the upper lobe of left lung associated with bronchiectasis, fibrosis and interstitial pneumonia. Diffuse purulent bronchopneumonia of both lungs. Fibrous pleural adhesions over upper lobe of left lung. Emphysema of both lungs. Marked atherosclerosis. Aneurysmal dilatation of the arch of the aorta. Hypertrophy of the right ventricle. Marked hyperplasia of the peribronchial and mediastinal lymph nodes.

Left lung. Weight 850 gm. The upper lobe had multiple cavities filled with dirty, foul-smelling pus. Several large, greenish areas of necrosis with beginning cavitation were seen. Smaller cavities filled with yellow creamy pus were found in the lower lobe. Interstitial pneumonia, diffuse bronchiectasis and extensive patches of necrotic, purulent bronchopneumonia occurred in both lobes.

Right lung. Weight 600 gm. Fibrous adhesions had formed at the apex and base. Purulent bronchitis and small areas of bronchopneumonia occurred in all lobes, and there were a few small abscesses in the lower lobe.

Smears from the abscesses in both lungs contained numerous spirochetes, and sections stained by the Levaditi method showed an abundance of organisms in the lung tissue.

Comment

From a study of these cases it seems evident that we are not dealing with a new clinical entity. The histories and necropsy findings suggest that certain serious pathologic conditions would have been present in these lungs if they had never become infected with spirochetes. It would seem, therefore, that there is always some primary destructive lesion followed by a secondary invasion by these organisms. In the study of lung abscesses and pulmonary gangrene, they have probably been overlooked because they stain poorly or not at all by ordinary staining methods. On the other hand it is probably of some significance that they are present in large numbers and are found more deeply seated in these lesions than other organisms. Just as Vincent's angina is a serious complication of acute tonsilitis, so the presence of these organisms in the lung tissue may mean more serious lung destruction by abscess or gangrene.

The organisms demonstrated in the above cases are variable enough in their morphology to correspond to all the types described by Castellani. The question then arises as to whether bronchial spirochetosis as an acute, subacute or chronic disease, is ever a clinical entity; that is, are these spirochetes ever the primary cause of such symptoms or, are they always secondary invaders? From a study of the chronic cases we find no evidence to oppose the belief that they are always secondary to some other infectious or destructive lesion. Symbiosis with other organisms probably plays no part in their growth, for they seem to thrive with a heterogeneous group of organisms in tissue that has been previously injured or is undergoing destruction. The next questions that arise are, is there any relationship between these spirochetes and the ones found in the human mouth, and is there any relationship between these organisms and the ones found in Vincent's angina? These questions we cannot definitely answer. The majority of those found in lung tissue are more slender and have more waves than those found in the mouth, although some do resemble the refringens type. Likewise these usually have more spirals than the Vincent type, but this type is encountered and organisms resembling fusiform bacilli are seen frequently enough to suggest that they are similar infections if not the same. The subject is open for further research to determine the relationship of the different types of spirochetes, and their significance in the various inflammatory processes in which they occur.

Conclusions

1. Spirochetes are frequently found in the sputum and lung tissue of patients with lung abscess or gangrene.

2. The frequent association of fusiform bacilli with these organisms suggests that they may be of the Vincent type, and not a definite entity as described by Castellani.

3. These organisms are readily demonstrated in the sputum when stained by gentian violet and Gram's iodine, or by the Warthin method, and in the tissues by the Levaditi or Warthin method.

4. Proper oral hygiene as a prophylactic measure, and treatment with arsphenamine in early recognized cases may be of benefit.

5. All forms of pulmonary spirochetosis probably represent a secondary infection. Their presence in large numbers, deeply situated in the tissue suggests some pathogenic influence.

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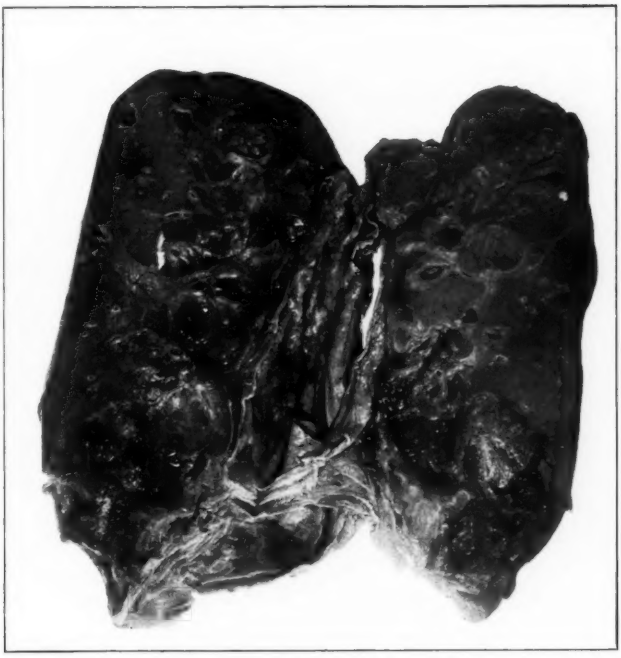
DESCRIPTION OF PLATES

Plate 72

Fig. 1. Case 1. Left lung. Pulmonary abscesses and interstitial pneumonia.
Fig. 2. Case 1. Right lung. Gangrenous abscesses.

Plate 73

Fig. 3. Case 4. Spirochetes in smear from gangrenous abscess of the lung.
Fig. 4. Case 3. Multiple abscesses and interstitial pneumonia.



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Smith and Rusk Pulmonary Spirochetosis

ADENOCARCINOMA OF THE UTERUS IN A RABBIT *

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Although rabbits are such common laboratory animals, the finding of spontaneous newgrowths among them, either benign or malignant, is quite rare. Bell and Henrici [1] were able to find reports of only thirty-five tumors. We have found reports of ten additional tumors. Of these forty-five tumors, twenty-four arose in the uterus. Stilling and Beitzke [2] state that in the course of their work from 1902 to 1913 they observed thirteen instances of tumors of the uterus. There were seven adenomas, four myomas and two apparently malignant tumors which infiltrated the wall of the uterus but gave rise to no distant metastases. Their attempts at transplantation into other rabbits failed to reproduce the tumor. Boycott [3] reports four instances of uterine tumors which arose at the site of placental remains, infiltrated the wall of the uterus and extended into the broad ligament. These tumors gave rise to no distant metastases. Other authors report single instances of uterine tumors, Wagner,[4] Selinow,[5] Katase,[6] Marie and Aubertin,[7] Leitch,[8] Shattock,[9] and Lack.[10]

The next most common site for tumors in rabbits is in the kidneys. These tumors have all been of the benign type giving rise to no metastases. None of these tumors was successfully transplanted into other rabbits. Seven such tumors have been reported.

There are reports in the literature of spontaneous carcinomas arising in the lung, stomach, accessory pancreas and in the breast, while sarcomas have arisen in the subcutaneous tissue, the omentum and elsewhere.

Recently W. H. Brown and Louise Pearce [11] have reported an instance of carcinoma developing at the site of a scrotal syphilitic infection. The tumor recurred after removal and finally metastasized to many organs leading to the death of the animal. This accidental occurrence led them to undertake an extensive experimental study of the relation between syphilitic infection and the incidence of cancer.

* Read before the section of Pathology and Bacteriology at the annual meeting of the California State Medical Association.

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Clinical History of the Rabbit

In 1919 the rabbit began its career as the pet of a family with several children. It remained apparently healthy until a few months before its exodus. At this time the animal became listless, lost its appetite and became greatly emaciated. A few days before death, blood was passed from what was taken to be the rectum. A necropsy was performed because it was suggested that there was a possibility of a malignant tumor of the bowel or the pelvic viscera.

It is of interest that the animal was a virgin female that presumably had experienced neither trauma, pregnancy nor infection, factors which might be considered as predisposing to the development of a malignant tumor. The animal was $4\frac{1}{2}$ years of age. On Sept. 23, 1924, the animal was killed by chloroform and immediately a necropsy was performed.

Necropsy Report

The animal is quite emaciated. The abdomen is greatly distended. Superficially no evidence of newgrowth is found. The peritoneal cavity contains about 500 cc. of straw-colored fluid. The lower abdomen is filled with a greatly distended bladder and two large irregular cystic masses which displace the intestines upward (Fig. 1). These masses conform to the Fallopian tubes which have been transformed into convoluted cystic masses measuring up to 16 cm. in diameter. These lobulations form a continuous cystic mass on either side with very thin walls but in the regions where the tubes join to form the uterus, the tissues are densely infiltrated with firm white nodules which completely replace the uterine tissue. The tumor tissue extends downward, infiltrating the vaginal walls but giving rise to no mucosal ulcerations. From the uterus the tumor extends into the bladder wall massively infiltrating the region of the trigone. As a result of this obstruction the bladder is greatly dilated to many times its normal size, measuring 15 cm. in diameter. The bladder wall is very thin, and extending from the region of massive infiltration are fine lines of tumor tissue invading the lymphatics. The bladder contains blood-tinged cloudy urine.

From the primary growth, the retroperitoneal, mesenteric, mediastinal and cervical lymph nodes are involved in the tumor invasion, forming a chain of masses from the pelvis to the neck varying in size from that of a bean to a lemon. On section the nodes can be seen to be composed of a uniform, opaque, whitish tissue evidently carci-

noma. In the region of the apex of the right lung is a mass, 2 cm. in diameter composed of tumor tissue involving a lymph node.

The right kidney is negative, but the left is moderately enlarged and, on section, reveals a marked hydronephrosis. There is a small rather recent blood clot in the pelvis. The ureter on this side is dilated and is partially obstructed where it enters the bladder.

Metastatic nodules of varying size are present in the liver and lungs.

The heart, spleen, osseous tissue, gastro-intestinal tract and central nervous system are entirely free of newgrowths.

Microscopic Examination

Uterus. There is very little endometrium present. On the inner surface is a small area lined with a single layer of columnar cells. Immediately beneath this area is an edematous fibrous tissue containing vessels which are congested and thin-walled. In this region there is a dense infiltration of lymphocytes. There are small groups of epithelial cells growing in strands which have large vesicular nuclei with finely granular basophilic cytoplasm. Some of these have undergone necrosis.

The muscular coat is extensively broken up into irregular bands by an infiltration of the newgrowth with an accompanying desmoplastic reaction. In general this newgrowth has a glandular arrangement with alveoli of varying sizes. These glandular structures are lined with a single layer of small cuboidal cells with deeply staining nuclei and with very little cytoplasm. In some areas the alveoli are filled with hyaline, pink-staining material. The cells lining these dilated alveoli are greatly flattened. The growth is definitely adenomatous in some areas, while in others it has a papillary form. A few mitotic figures are present.

Fallopian Tube. The inner surface is lined with a layer of very flat epithelial cells. The tube is so stretched that the normal convolutions are obliterated. The tissue beneath the lining layer is a vascular and edematous stroma. This layer and the remaining coats of the tube are infiltrated with a newgrowth of epithelial cells which present the glandular and papillary arrangement much like that seen in the uterus. Mitotic figures are present. Sections of the tubes taken elsewhere show the wall to be greatly thinned in places and composed mainly of a hyaline fibrous material.

Bladder. The bladder wall is greatly thinned. The mucosal lining is intact and is composed of two to three layers of epithelial cells. The mucosa appears normal but the submucosa is very vascular and congested. The blood vessels are thin-walled. There are several clumps of cancer cells which have infiltrated the submucosa almost to its epithelial lining. These cells are enclosed in small spaces lined with endothelial cells. These spaces undoubtedly represent dilated lymphatics.

The muscular coat of the bladder is diffusely infiltrated with the newgrowth. There is a rather dense desmoplastic reaction accompanying the tumor. There is a tendency for some of the central cells to undergo necrosis.

Lung. The lung tissue is normal except for a slight amount of congestion. Scattered through the tissue are metastatic tumor nodules. Each of these is composed of a narrow rim of well preserved cancer cells in gland-like arrangement. Within this narrow rim of growing tissue the cells gradually lose their staining power and become necrotic. Microscopic collections of cancer cells are found elsewhere in the lung.

Kidney. There is a marked dilatation of the calices and the tubules. This distension is greatest in the collecting tubules and gradually decreases in the cortex. The epithelium of the dilated tubules is flattened. Between the tubules there is a marked interstitial proliferation of fibrous tissue. The pelvis contains a small amount of fibrino-sanguinous exudate. There are no cancer cells present.

Lymph Node. The architecture of the retroperitoneal lymph nodes is completely distorted due to an extensive infiltration by the newgrowth, which is accompanied by a rather dense fibrous stroma and assumes a medullary type of growth. Several dilated spaces lined with endothelium, evidently dilated lymphatic vessels, contain clumps of epithelial cells. Some of these cells are well preserved and have mitotic figures.

Attempts at Transplantation

Immediately following the autopsy attempts were made to transplant the tumor into six other rabbits. The methods used were the subcutaneous and intraperitoneal injections of finely-chopped tumor mixed with warm normal saline solution. Four of these rabbits subsequently died of infection, while two were killed with chloroform

several months after inoculation. None of the rabbits showed tumor growth.

W. H. Brown and Louise Pearce were not successful in transmitting a cancer to other rabbits until they made an emulsion of the tumor cells and then injected them into the testicles of other rabbits. The emulsion was made by grinding some of the tumor tissue in a mortar and adding normal saline solution, 0.5 cc. being used as the dose. They succeeded in transmitting the tumor in this way to a number of rabbits.

Summary

A virgin female rabbit, 4½ years of age, developed a spontaneous carcinoma of the body of the uterus with extension into the tubes, bladder and vaginal walls. Extensive metastases were formed in the retroperitoneal, mesenteric, mediastinal and cervical lymph nodes, in the liver and lungs.

The carcinoma in general maintained an adenomatous or papillary adenomatous type but in some areas it grew in a medullary form. In some areas the tumor alveoli contained a colloid-like material. The tumor cells could be seen invading the dilated lymphatics of the bladder and lymph nodes.

All efforts at transplantation failed to reproduce carcinoma.

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DESCRIPTION OF PLATES

Plate 74

Fig. 1. Shows the viscera at the time of autopsy. The bladder is greatly distended and shows dilated lymphatics in its wall containing carcinoma. The irregular lobulated cystic masses in the lower abdomen are the Fallopian tubes. Between the bladder and tubes, the uterus can be seen infiltrated with carcinoma which extends into the bladder and tubes. Metastases may be seen in the omentum, liver, right lung and at root of neck.

Plate 75

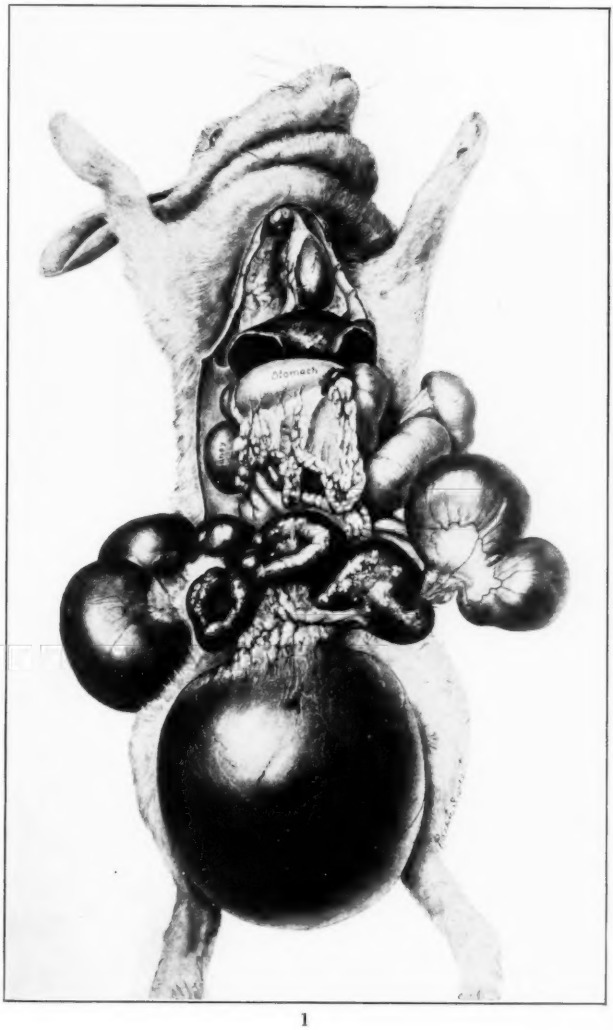
Fig. 2. Microscopic picture of the uterus (low power) showing glandular type of growth with hyaline material within the alveoli. Also shows muscle bands between the cancer nodules.
Fig. 3. Uterus under 4. mm objective. Note the smooth muscle cells between the aveoli.

Plate 76

Fig. 4. Bladder shows cancer cells in dilated lymphatics.
Fig. 5. Lymph gland shows invasion of the gland with carcinoma growing in solid masses. Mitotic figures are present.

Plate 77

Fig. 6. Fallopian tube shows invasion of the entire wall with a papillary-adenomatous type of newgrowth.
Fig. 7. Lung showing metastatic nodule with central necrosis.



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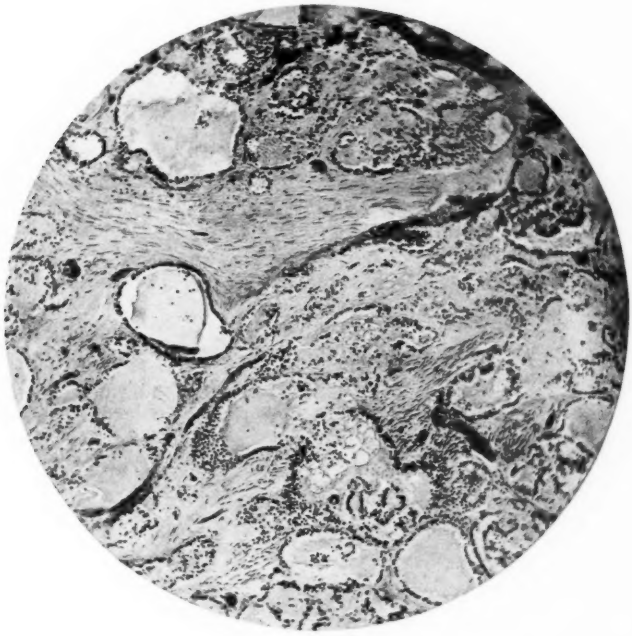

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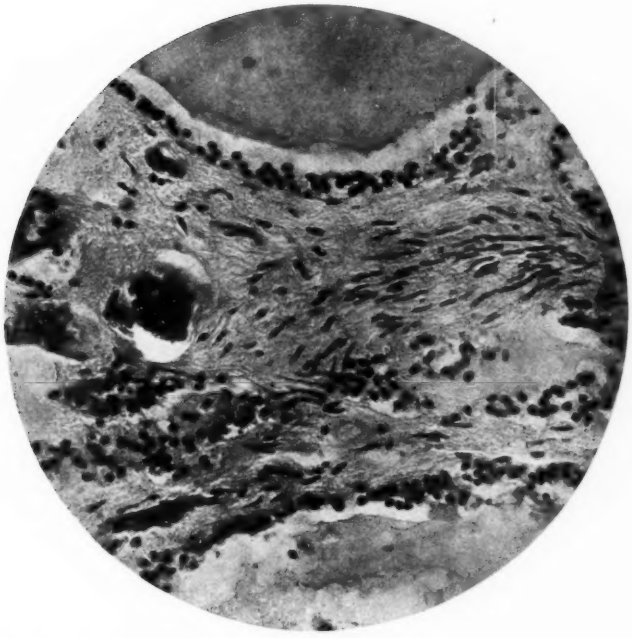

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Rusk and Epstein Adenocarcinoma of the Uterus in a Rabbit

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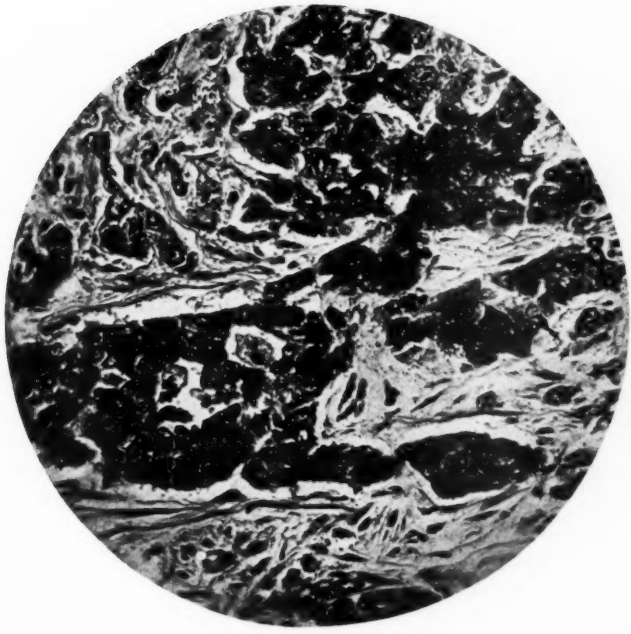

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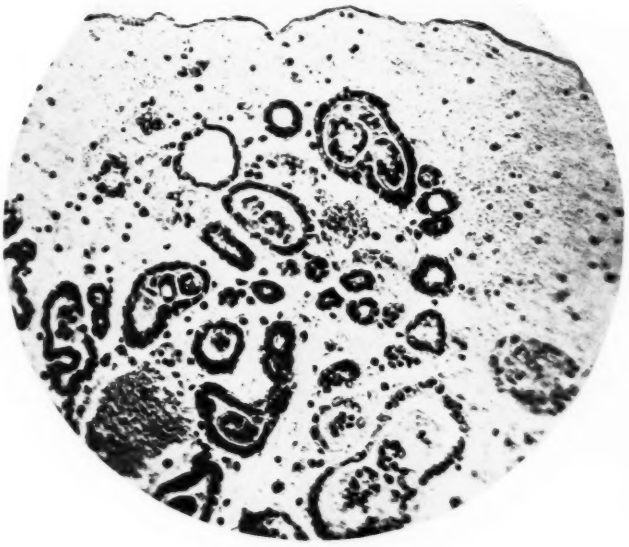
Rusk and Epstein Adenocarcinoma of the Uterus in a Rabbit



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Rusk and Epstein Adenocarcinoma of the Uterus in a Rabbit

TUBERCULOSIS OF THE TONGUE *

WITH A CASE REPORT

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The tongue appears to possess considerable immunity against invasion by the tubercle bacillus, and while tuberculosis is undoubtedly more frequently encountered in this organ than the modern textbooks would indicate, yet a review of the literature suggests that this particular manifestation of the disease is infrequently reported.

To Portal[1] is usually given the credit for describing the first case of tuberculosis of the tongue. Portal's paper was published in 1804, and since that time practically every decade has seen a sprinkling of cases appearing in the literature.

It is not the intent to present here a complete bibliographic review of tuberculosis of the tongue. To attempt this would result in a duplication of effort since such a digest is available in the excellent paper by Scott[2], who reviews the literature to 1916 with a collection of 231 cases, 26 of which were primary. Scott also contributes one case. The patient was a soldier aged 32 years. The lesion on the tongue existed for four years, during which time the man was repeatedly examined for pulmonary tuberculosis with negative results. It was later determined that both lungs possessed a tuberculous involvement. Since the publication of Scott's paper a number of new cases have been reported and a brief summary of these follows.

Durante[3] discusses the pathologic and surgical aspect of the disease and describes five cases from material in the Mayo Clinic. Three of the cases were males aged 24, 40 and 46 years respectively, while the ages of the females were 30 and 46 years respectively.

Three of the cases were associated with the respiratory type of the disease, while in two it was not possible to determine any involvement except the tongue. Durante ends his paper with a formidable list of references on the subject.

White and Marcy[4] describe a case in a man who had suffered from pulmonary tuberculosis for about four and one half years previous

* Received for publication January 24, 1927.

to the development of the tongue lesions. This case is of particular interest because of the fact that the authors were able to effect a healing of the tongue lesion by repeated injections of Koch's old tuberculin.

Besley[5] reports a case in a male age 52 with active pulmonary tuberculosis with sputum showing the bacilli. Besley's case had previously been erroneously diagnosed as tongue carcinoma.

Taddei[6] reports a case in a woman 52 years old with a tongue lesion of two months duration. The tongue lesion was perhaps primary since there was no other evidence of the disease. The nodule ulcerated and was removed, and a guinea-pig inoculation with some of the infected tissue was successful in demonstrating the true nature of the infection.

Handfield-Jones[7] gives a thorough presentation of the pathologic and surgical aspects of the disease, and presents five cases, three males and two females. One patient, a male, who was affected with pulmonary tuberculosis had a history of having bitten his tongue seven weeks before ulceration appeared. Three of Handfield-Jones's cases had unmistakable pulmonary tuberculosis, with the tongue lesion appearing secondary, while in two there was no evidence of primary tuberculosis elsewhere. The ages of the males were 59, 28 and 42 years respectively, while the females were 37 and 42 years respectively. The average age of this series was 41.

Fantozzi[8] describes an interesting case in a woman age 52, who developed a tuberculous lesion of the tongue, about one month after an injury to the zygoma region which was incurred by falling to the floor.

Morrow and Miller[9], in a splendid paper, report sixteen cases in which all but one were males. Of the sixteen, fifteen were secondary while one was perhaps primary. These authors also present a table showing that 40 per cent of the patients in their series were between the ages of 30 and 40, the average age of all patients being 41.7 years. They also mention as a possible explanation of the high incidence of the disease in the male, the fact that he is more subject to trauma of the tongue from carious teeth, pipe-smoking and the frequent practice of putting metallic objects like nails into the mouth.

The sixteen cases reported were from a clinic of 1444 tuberculous patients during a period of four years. This would give the percentage of incidence at a little less than 1 per cent.

Finney and Finney[10] report fifteen cases of which thirteen were males and two females. Five were thought to be primary. Three of the cases were mistaken for carcinoma of the tongue and were operated upon for this condition. The majority of these authors' cases were in the forties, the average for all being 41.7 years.

Henry[11] describes four cases, all males, suffering from pulmonary tuberculosis, with the tongue lesions secondary. The sputa of all four patients contained tubercle bacilli in considerable numbers. The ages of Henry's cases were 54, 29, 24 and 40 years respectively. The average for all was 37 years.

Bass [12] gives a description of the disease together with an extensive bibliography, and presents two cases, one a physician age 43 years, male, with a doubtful history of tuberculosis elsewhere; the other a

TABLE 1

Summary of the Cases Reported above

Author	Number of cases	Sex		Age average	Lesion	
		Male	Female		Primary*	Secondary
Scott [2]	1	1	0	32	0	1
Durante [3]	5	3	2	37	2	3
White & Marcy [4]	1	1	0	30	0	1
Besley [5]	1	1	0	52	0	1
Taddei [6]	1	0	1	52	1	0
Handfield-Jones [7]	5	3	2	41	2	3
Fantozzi [8]	1	0	1	52	1	0
Morrow & Miller [9]	16	15	1	41.7	1	15
Finney & Finney [10]	15	13	2	41.7	5	10
Henry [11]	4	4	0	37	0	4
Bass [12]	2	2	0	54	1	1

* The word primary is used here in a restricted sense, meaning that in most instances it was impossible, from the available information to determine primary lesions elsewhere.

male age 66 years having in addition to the tongue lesion a tuberculous adenitis. This second case possessed no history or clinical evidence of tuberculosis except the tongue and submaxillary lymph nodes, although previously, two wives of this man had succumbed to the disease.

The fifty-two cases reported above added to the 231 cases previously collected by Scott [2] give a total of 283 cases of tuberculosis of the tongue which have been reported to date. Perhaps the actual

total is slightly above this figure since it is difficult for any one author to be quite sure of having reviewed all the literature on such a subject as this.

From the above chart we see that forty-three of the fifty-two cases were males while only nine were females. The age of greatest incidence is interesting in that the majority of the cases were in the early forties, with the average for the entire fifty-two cases being 42.7 years. The tongue form of this disease is rarely primary, there being but thirteen of the fifty-two cases which might be classified as such. However, as mentioned in the footnote, it is decidedly difficult to classify tongue lesions as primary, unless the possibility of tuberculous infection elsewhere is ruled out by a necropsy or other suitable procedures. Using Scott's [2] figures again, we find that out of the total 283 cases there are only thirty-nine which might be classed as primary. This emphasizes the importance of a thorough search for tuberculosis in some other part of the body when the infection is found in the tongue.

Morrow and Miller's[9] statement that they found a little less than 1 per cent of a tuberculous population of 1444 affected with the tongue form of the disease, is very suggestive that tuberculosis of the tongue is considered to be rare. It might be suggested that if special attention were given to this organ during the physical examination of the living, and at necropsy of those dying of tuberculosis, the reputed rarity of this form of the disease might be disproved.

The strikingly high percentage of males over females is remarkable and has not, in my opinion, been logically explained by those who have studied and described this form of tuberculosis. Dental caries and sharp jagged teeth have been attributed as factors influencing the high incidence in the male, but without sufficient proof. I have been informed by dentists that jagged points are seen more frequently in men than in women due perhaps to chewing of tobacco which causes an uneven wearing of the teeth. The sharp points may in turn cause abrasions. However, it cannot be properly assumed that all or even the majority of men affected with tuberculosis of the tongue have been chewers of tobacco. On the other hand, it is probably correct to assume that none of the women affected was addicted to this habit. The evidence here while perhaps suggestive is not conclusive and certainly is not sufficient to explain the matter in its entirety. Some mention that men in certain vocations, such as car-

pentry and shoe repairing, frequently carry in their mouths metallic objects such as nails, and, of course, the likelihood of trauma that would permit the entrance of the tubercle bacillus into the mucosa and underlying tissues is considerable. This explanation of the high incidence of tuberculosis of the tongue in the male is entirely inadequate when one considers that carpenters and shoemakers were conspicuous by their absence in the case histories reported.

While I feel that the majority of the tongue lesions arise from the inoculation of a break in the continuity of the mucosa of the organ, usually in the nature of a traumatism, I fail to recognize any factor of a material kind that sufficiently accounts for the predominance of cases in the male.

In a matter of this kind, one is tempted to suggest as a hypothesis a sex susceptibility which may be possessed by certain males, and to a lesser degree or not at all by females. The apparent natural immunity of striated muscle to the tubercle bacillus has been commented upon many times, and is perhaps the explanation in part at least of why the tongue is so rarely involved. The proposition of susceptibility is of sufficient importance to be considered at least a contributory factor in those in which the tongue is affected. The influence of susceptibility and immunity must remain a matter of conjecture since adequate proof at present is impossible to assemble.

There can be no doubt that the tongue of all sufferers of open pulmonary, pharyngeal and laryngeal tuberculosis is continuously exposed to secondary infection by the bacilli laden secretions with which the tongue is in constant contact. The wonder is that it succumbs so infrequently to invasion.

In a few instances the cases possessed a history of tongue lacerations by self-inflicted bites. Such trauma is common even in the non-tuberculous as the personal experience of all of us could testify. No doubt many bite wounds that later became sites of tuberculous lesions were considered inconsequential and promptly forgotten. As a consequence perhaps bite wounds are not mentioned as frequently as their true position in the pathogenesis of the disease warrants.

Some have placed, I think, undue emphasis upon the effect of pipe-smoking as a contributory factor in causing the tongue lesion. From the evidence presented in the reported cases, it is difficult to look upon pipe-smoking as playing any rôle whatever in the pathogenesis

of tongue tuberculosis, or at the most the influence of this habit must be negligible.

It is likewise difficult to account for the age incidence. The ages of the majority of the reported cases have been in the early forties. This, together with the fact that most of the tongue lesions have been secondary to a pulmonary involvement, would suggest that the tongue is more prone to attack in the later stages of the disease and at a time when the resistance may be diminished.

Report of a Case

The patient, auto salesman, age 36, in the practice of Dr. W. A. Kickland, had suffered with pulmonary tuberculosis since he was 22 years of age (fourteen years). Four months before the tongue lesion developed he broke a tooth and bit his tongue with the remaining sharp fragment. A painful nodule appeared in the same area, which was on the right lateral surface, about one inch from the tip. Soon after the appearance of the nodule, another physician removed a portion, and reported the presence of acid-fast bacilli. The lesion was then cauterized with silver nitrate. The nodule persisted however, and two months later the lesion was removed surgically and the wound electrically cauterized. The area has been treated at intervals with the ultraviolet ray, and at the present time the patient appears to be making a satisfactory recovery, although it is probably too early to venture an ultimate prognosis.

Pathology. Sections were prepared from the tissue which was removed at the operation, and stained with hematoxylin and eosin. Others were stained with hematoxylin and Ziehl-Neelson's carbol fuchsin for the purpose of demonstrating any acid-fast organisms present. Sections were cut at different planes to facilitate a comprehensive study of the material.

Immediately beneath the mucosa there was a large irregular area of early ulceration or necrosis quite devoid of giant cells, and with no evident attempt at encapsulation (Fig. 1). Adjacent to this zone the muscle for a considerable depth was involved in a typical tuberculous process. The lesion consisted of tubercles which occupied the muscle bundles or fascicles and were rather sharply separated from each other by the remains of the perimysium (Figs. 2 and 3). Many of the muscle bundles contained only a remnant of muscle fibers which

were badly distorted due to the pressure exerted by the adjacent tubercles. The intermediate areas of some of the tubercles showed considerable accumulations of endothelial leucocytes with an occasional lymphocyte. In the extreme depths of the tissue a few muscle bundles had been replaced by collections of epithelioid (endothelial) cells without giant cell formation. These accumulations appeared to be very early tubercles, many of which were separated from the great zone of tubercles by bundles of normal muscle fibers indicating that perhaps the mode of extension of the infection was at least in part hematogeneous. The tubercles appeared to be of the same duration and fairly young as none showed caseation or calcification. A few of the tubercles showed only one giant cell, but the majority possessed two or three, and many five to seven (Fig. 3). Blood channels in the affected portion were few, and the perivascular infiltration noted by others was not observed.

Acid-fast bacteria of the morphology typical of the tubercle bacillus were demonstrated in a few of the sections stained with hematoxylin and carbol fuchsin. The organisms were located within the body of the giant cells and were very few in number.

Discussion

From the history of the above case together with the pathologic findings there can exist no reasonable doubt that we were dealing with a case of tuberculosis of the tongue. The pathology also bears out the history that the lesion was of recent origin. The multiplicity of the tubercles, the majority of which were apparently of the same age, would indicate a rather massive inoculation, and a general susceptibility of the muscular fibers to the infection. While the original infection of the tongue undoubtedly resulted as a direct inoculation of the bite wound by the bacilli, there is some reason to think that part of the subsequent extension has been by way of the local blood vessels. It would be difficult to account for the young tubercle formations in the depth of the tissue by any other manner. The extension in the deeper portions by continuity would appear to be quite improbable.

The multiplicity of the tubercles and their widespread distribution even up to the edge of the line of incision, would cause one to doubt the likelihood of all the diseased tissue being removed. Consequently

the use of the cautery at the time of the operation and the subsequent employment of the violet ray were considered proper procedures.

Summary

1. A review of the literature available since 1916, dealing with tuberculosis of the tongue is presented.

2. About fifty-two cases have been reported during the past ten years. These added to those previously reported bring the total of cases up to 283.

3. The assembled data indicate that the disease is nearly five times as frequent in the male as in the female.

4. The average age incidence of the fifty-two cases reviewed is 42.7 years.

5. An original case is described.

The writer is indebted to Dr. W. A. Kickland, who kindly furnished the information necessary for the history of the case reported.

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DESCRIPTION OF PLATES

PLATE 78

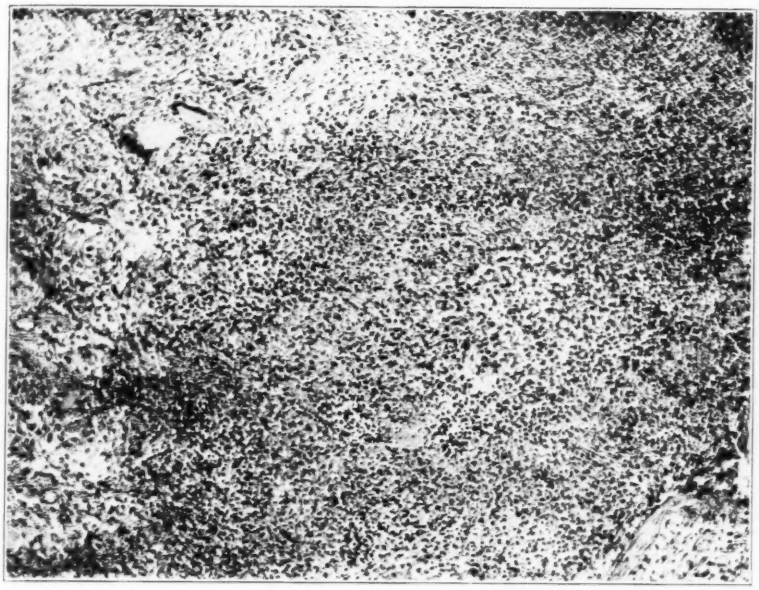
FIG. 1. Tuberculosis of the Tongue. Low power view of an area in the submucosa showing ulceration.

FIG. 2. Tuberculosis of the tongue. Low power photomicrograph of a tubercle occupying the space normally occupied by a bundle of muscle fibres. Note the many giant cells and the sharp separation of the tubercle from the surrounding tissue.

PLATE 79

FIG. 3. Tuberculosis of the tongue. Low power photomicrograph of a tubercle showing a large number of typical giant cells.

FIG. 4. Tuberculosis of the tongue. High power photomicrograph of a giant cell.



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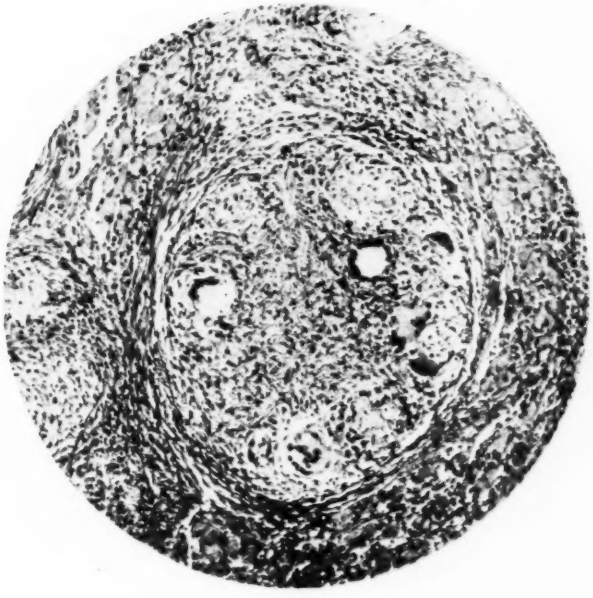
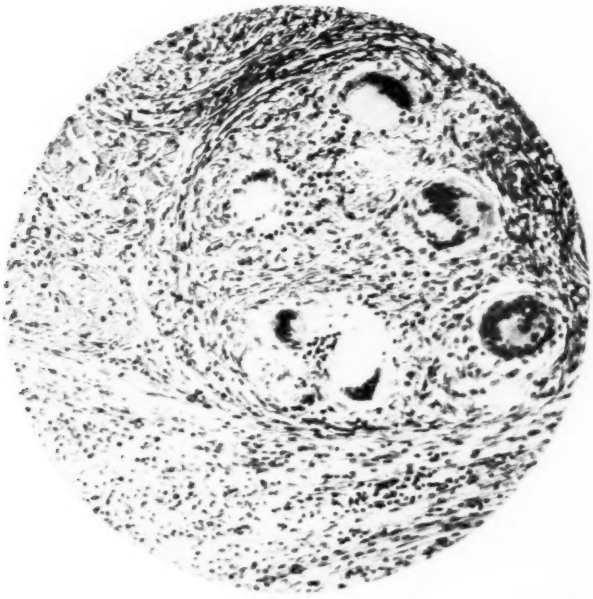


2

Feldman Tuberculosis of the Tongue



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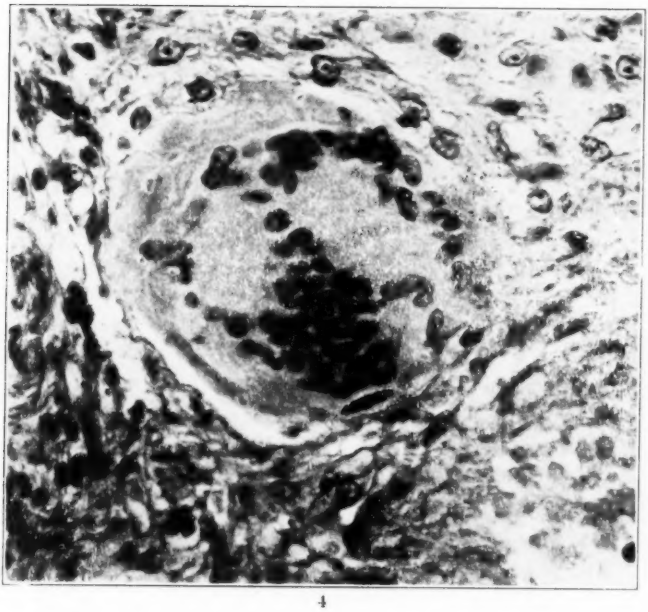

4

TRANSPLANTATION AND INDIVIDUALITY DIFFERENTIALS IN INBRED FAMILIES OF GUINEA-PIGS *

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In a series of papers one of us analyzed the reactions of the host against auto, syngenesio, homoio and heterotransplants.[1] Definite relations were found between the intensity of the lymphocytic and connective tissue reactions and the genetic relationship between host and donor. The similarity or differences between the individuality differentials of host and transplant largely determined the effects of the transplantation. These relations between individuality differentials decided whether the substances given off by the transplant affected the host as normal auto substances or as toxins of various intensities (syngenesio, homoio, heterotoxins). In the case of autotransplantation, there exists an identity of individuality differentials. The intensity of the reactions appearing after transplantation furnished a quantitative measure of the similarity or difference between individuality differentials. It was to be assumed that through long-continued inbreeding the individuality differentials among the members of the inbred family would gradually become more and more alike. In addition to these primary factors affecting the individuality differential there were active secondary factors of a nongenetic character.

Under these conditions it was of interest to extend these experiments to inbred strains, and this paper deals with the results obtained in the exchange of tissues in families of guinea-pigs which have been inbred in the United States Department of Agriculture since 1906. Five different families have thus been developed through continued sister and brother matings (2, 13, 32, 35 and 39). However, the degree of homogeneity in the various families differs.[2] In addition, control experiments were made in which tissues were exchanged with members of a B group. In this group, originally derived from the same stock as the inbred families, matings as close as those between second cousins have been avoided. These latter

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experiments approached therefore homoiotransplantations.* Each guinea-pig was checked by a number assigned serially and given here in brackets. Each inbred mating is designated by three figures. The first is the family number (2, 13, 32, 35 and 39); the second is the number of generations of brother-sister mating since the foundation (O) mating of the family; while the third is arbitrary. Experiments 2N, Y and 35I involve only families 2, 13 and 35 respectively but are not brother-sister matings. Experiment CO is composed of crosses between inbred families.

I. Transplantations in the same Family, Host and Donor not Being nearly Related

Series A. *Experiments in which the examination took place within two months following transplantation.* Weight of guinea-pigs in most cases varying between 200 and 500 gm.

1. From guinea-pig 32-17-10 (15611) to guinea-pig 32-19-8 (321), *40 days*. Both thyroid and cartilage transplants are like perfect autotransplants, as far as structure and lack of increase in lymphocytes and connective tissue are concerned. Grade 6.

2. From 2–17–10 (15959) to 2–17–5 (15694) *50 days*. Thyroid, parathyroid, and cartilage autotransplants. Grade 6.

3. Y–4 $\left\{\begin{matrix}13\text{–}20\text{–}2\\13\text{–}19\text{–}5\end{matrix}\right\}$ (16039) to Y–7 $\left\{\begin{matrix}13\text{–}20\text{–}2\\13\text{–}19\text{–}6\end{matrix}\right\}$ (16029) *40 days*. Syngenesio-reaction. Grade 5. Thyroid shows auto-structure, but there is much lymphocytic infiltration in certain areas of the center. From here lymphocytes penetrate between acini toward periphery and surround some acini. Around a vessel traversing the ring of acini are lymphocytes. In other places the center is free from lymphocytes. Cartilage transplant with areolar tissue is well preserved; but there is some increase of connective tissue around the cartilage and an incomplete mantle of lymphocytes.

4. From 32–17–11 (15575) to 32–17–8 (15634) *40 days*. Thyroid autotransplant. Grade 6.

5. From 13–19–9 (15649) to 13–20–5 (15563) *35 days*. Syngenesio-reaction. Grade 5. Thyroid with auto-structure; parathyroid well preserved, but intense lymphocytic infiltration around and in parathyroid, and in places in the periphery of the thyroid. In various

* These figures have been mentioned in a previous paper.

places small parts of thyroid have been destroyed by lymphocytes which accumulate around vessels. Normal cartilage surrounded by areolar and fat tissue, with only a slight increase in connective tissue and lymphocytes.

6. From 13–20–5 (15563) to 13–20–13 (15782) *31 days*. Syngenesio-reaction. Grade 5. Lobes of thyroid were transplanted (parts of the transplants were used for retransplantation). Structure of autotransplants, but in center there is moderate, although distinct, lymphocytic infiltration; lymphocytes penetrate also between acini and surround and destroy some of them. Moderate increase of connective tissue between acini.

7. From 32–18–9 (20865) to 32–19–9 (16900) *38 days*. Resembles autotransplant. Grade 6.

8. From 2–18–4 (15693) to 2–16–16 (15590) *49 days*. Syngenesio-reaction. Grade 5. Well preserved thyroid. Considerable lymphocytic infiltration penetrating a little toward periphery and also into parathyroid. Slight connective tissue increase around cartilage. Slight increase of lymphocytes in areolar tissue.

Eight experiments constitute this series. In four cases an auto-reaction was obtained; in four cases a syngenesio-reaction. In the latter cases the transplant first began to develop as an autotransplant, but later, lymphocytes began to invade the transplant and even connective tissue penetrated between some of the acini. We have, therefore, in these cases to deal with syngenesio-reaction. Auto-reactions were found 38, 40 and 50 days after transplantation, syngenesio-reactions 30–50 days after transplantation. Auto-reactions were obtained in families 32 and 2, syngenesio-reaction in family 13, and in one case in family 2. There can be no doubt that a difference existed in the constitution of the individuality differentials in the members of families 13 and 2 which showed syngenesio-reaction. In the two cases in family 32 and in one case in family 2 the reactions did not indicate a difference in individuality differentials, but this does not necessarily exclude the possibility that such a discrepancy would not have been revealed if we had extended the experiments over a still longer period. On the other hand we can state definitely that the members of the same inbred family are more nearly akin to each other as far as the constitution of their individuality differential is concerned, than in ordinary guinea-pig families where brothers are related to each other.

SERIES B. *In a second similar series the examination took place at a still later date in the majority of cases.* In these experiments the weight of the animals was generally greater, in accordance with the greater age of these guinea-pigs.

1. From 13–20–5 (15563) to 13–20–13 (15782) *5 months, 12 days.* Transplanted thyroid was not found.

2. From Y–7 $\left\{ \begin{matrix} 13–20–2 \\ 13–19–6 \end{matrix} \right\}$ (16029) to 13–20–13 (15782) *5 months, 12 days.* In this case there is a decided syngenesio-reaction. A part of the transplanted thyroid was preserved and the acini contain well formed colloid. But there is much lymphocytic infiltration. The lymphocytes penetrate everywhere between the acini and even into the epithelium and destroy it. There is also some increase of fibrillar connective tissue around some acini. Lymph vessels are filled with lymphocytes. Large masses of these cells surround and encompass acini which have lost their colloid. Grade 4.

3. From 2N–27 (18040) to 2–17–14 (18450) *102 days.* Thyroid and cartilage were perfect autotransplants. Grade 6.

4. From 13–19–15 (18317) to 13–21–7 (18493) *102 days.* Thyroid, parathyroid and cartilage with surrounding tissue resemble autotransplants, except that in one place in the cartillage transplant there was a slight collection of lymphocytes around a vessel; this latter condition may still come within the range of autotransplant. Grade 6 or 5.75.

5. From 2–16–16 (15590) to 2N–26 (15673) *104 days.* Autoreactions in thyroid and cartilage transplant. Grade 6.

6. From 32–19–8 (15634) and 32–19–8 (15633) to 32–19–9 (16900) *37 days.* Two thyroids, and parathyroids transplanted. Auto-reaction, except that in one place in the center there is a considerable lymphocytic infiltration. Grade 5.60.

The six experiments of this series confirm and extend the conclusions of the first series. Experiment 1 is uncertain as to the interpretation of the results, no thyroid transplant having been found after 5 months, 12 days. It is possible that the transplant has been destroyed after such a long period, especially considering the fact that transplantation was carried out in family 13, in which there is apparently a greater tendency on the part of the host to react against the transplant from another individual of the same family. A marked reaction was obtained in family 13 in the second case follow-

ing a transplantation extending over a long period of time. In the fourth case, on the other hand, we find after almost three and a half months a condition approaching that found in autotransplants. It is, of course, to be assumed that in certain combinations of animals of this family the individuality differentials may happen to be identical or almost identical. In two experiments in family 2 the results indicated an identity, or a condition almost approaching identity, of the individuality differentials. In one experiment in family 32, extending over 37 days, the result at present is similar to that obtained in autotransplantation, but there is some indication that with an extension of the experiment, the differences between the individuality differentials would come out definitely. These experiments then demonstrate the lack of identity of individuality differentials in family 13 and probably also in family 32. The pedigree analysis indicates that the large number of reactions not auto in family 13 is probably due to the large number of experiments in which host and donor had less than 10 generations of common inbreeding. At the same time these experiments prove that the similarity of individuality differentials is still greater than appeared in the first experiment, inasmuch as even after relatively long periods of time the conditions obtained may still correspond to those found in autotransplantation. And even where, as in some cases in family 13, a definite discrepancy between the individuality differentials has been found, the latter are still much more closely related than the average of the individuality differentials of brothers in non-inbred families of guinea-pigs.

These experiments demonstrate anew the great sensitiveness of the lymphocytic reaction which may appear at a very late stage of transplantation and then exert a destructive effect in case the syngenesio toxins are very weak. There may be also associated with this lymphocytic reaction a secondary slight proliferation of the connective tissue in the transplant.

SERIES C. *Transplantation of tissues from a hybrid between two inbred species to another hybrid of similar constitution.*

1. From C–O–297 $\left\{\begin{matrix} 2\text{–}17\text{–}8 \\ 35\text{–}23\text{–}10 \end{matrix}\right\}$ (17093) (320 gm.) to
C–O–234 $\left\{\begin{matrix} 2\text{–}13\text{–}7 \\ 35\text{–}16\text{–}20 \end{matrix}\right\}$ (15880) (632–682 gm.)
31 days. Thyroid with auto-structure; in one place in center there is a considerable lymphocytic infiltration; the lymphocytes infiltrate

also parathyroid and surround some acini of thyroid. Cartilage similar to autotransplant; but there is in places in the surrounding areolar and fat tissue a slight increase in connective tissue with some lymphocytes around vessels. Near such places in fat tissue are found some giant and epithelioid cells; elsewhere fat tissue is free from the latter structures. Grade 5.

2. From C–O–285 $\left\{\begin{matrix}35\text{–}21\text{–}2\\32\text{–}15\text{–}15\end{matrix}\right\}$ (15942) to C–O–240 $\left\{\begin{matrix}32\text{–}16\text{–}9\\35\text{–}16\text{–}11\end{matrix}\right\}$ (15943) *35 days*. Thyroid and cartilage resemble autotransplant. Grade 6. The number of experiments in this series is too small to allow of any definite conclusion.

SERIES D. *Successive transplantation in the same family.* In a few cases, pieces were transplanted to an animal of the same strain, and after these pieces had been in the first host for some time, they were removed and retransplanted into a second host belonging likewise to the same strain. After a certain period these retransplanted pieces were taken out and examined microscopically.

1. First transplantation. From 13–20–5 (15563) and from Y–7 $\left\{\begin{matrix}13\text{–}20\text{–}2\\13\text{–}19\text{–}6\end{matrix}\right\}$ (16029) to 13–20–13 (15782) *31 days.*

Second transplantation. 13–20–13 (15782) to 13–18–9 (16174) *23 days*. Thyroid shows structure of autotransplant: a ring of acini with a small amount of connective tissue in center. The majority of acini contain colloid, others do not. Between acini there is fibrillar connective tissue with much lymphocytic infiltration; a number of acini have been destroyed by lymphocytes or connective tissue. The lymphocytic infiltration is in places moderate and in others more marked, though not overwhelming. Lymphocytes invade some acini. Grade 3.75. Here in family 13 is a very decided syngenesio-reaction. The piece was in a strange host during a period of *54 days*. The marked reaction found in this case is in accordance with the fact that the second host, 16174 (13–18–9) had only 7 generations of brother-sister ancestry in common with either of the donors.

2. First transplantation. Cartilage transplanted from guinea-pig 13–21–19 (20898) to 13–20–17 (20761) *6 days*.

Second transplantation, 13–20–17 to 13–23–1 (20986) *7 days*. Duration of experiment *13 days* altogether. The original cartilage

though showing areas of necrosis, also shows some newly-formed perichondrial cartilage. The fat tissue which is preserved is invaded in places by connective tissue. There is much fibrillar connective tissue around the cartilage. In the absence of a lymphocytic infiltration it is doubtful whether the connective tissue increase is to be considered as a specific reaction against the transplant.

3. First transplantation. Four thyroids from two guinea-pigs 32–19–8 (15634 and 15633) to guinea-pig 32–19–9 (16900) *37 days*. The specimen is like an autotransplant or nearly so, with perhaps a slight increase in connective tissue.

Second transplantation, 32–19–9 (16900) to brother 32–19–9 (16899) *4 months, 9 days*. Thyroid is like an autotransplant with low to medium-sized epithelium of acini which are close together and contain solid, much retracted colloid. There is a small amount of fibrous tissue in the center. The transplant is surrounded by fibrous tissue. The parathyroid is negative, although in the capsule around the parathyroid there are some lymphocytes. Grade 6 or 5.75. In this case the second transplantation was made into the brother of the first host. The total duration of experiment 2 was five and one-half months, and yet no definite reaction against the tissue has occurred. This demonstrates the great similarity of individuality differentials in this case. These experiments further demonstrate the feasibility of serial transplantations with as sensitive an organ as the thyroid gland, provided the individuality differentials of the hosts are similar to those of the donors.

SERIES E. *Multiple simultaneous transplantations in the same family, with examination within 6 weeks after transplantation.* In this and in the following series the variety of organs which were transplanted was enlarged; in addition to thyroid, parathyroid, and cartilage, such organs as spleen, adrenal, liver, pancreas, and bone, were also transplanted. In the first experiments four thyroids from two different donors were transplanted. It was of interest to test how more sensitive pieces of organs or tissues would behave under the favorable conditions in which the individuality differentials in host and donor are so similar to each other. Five experiments were made in this series.

1. 32–19–8 (15634) and 32–19–8 (15633) four thyroid lobes to 32–19–9 (16900)

37 days. Two thyroids examined. Both lobes behave like or almost like autotransplants, but there are collections of lymphocytes in lymph vessels and in various places in the fibrous tissue around the thyroid transplant. Grade 5.60.

2. Liver, spleen and pancreas from 13–19–9 (15648) to 13–18–10 (16787) *36 days*. The spleen shows lymph follicles in its periphery, pulp with blood sinuses and strands of hyaline connective tissue probably corresponding to trabeculae. Perhaps in the periphery of the capsule there is some lymphocytic infiltration. The lymph follicles have larger cells in their centers and smaller cells in the periphery. In the liver, the bile ducts are preserved and are actively proliferating, showing mitoses. They are surrounded by a fibrous capsule with slight lymphocytic infiltration. The liver tissue is well preserved, and apparent transitions between bile ducts and liver tissue are visible. Mitoses seem to occur in the liver cells. There are small collections of lymphocytes here and there in the liver tissue. The pancreas shows only fat tissue with an epitheloid, giant cell and lymphocytic infiltration. Grade about 5.50.

3. Adrenals from Y–4 $\left\{\begin{matrix}13\text{–}20\text{–}2\\13\text{–}19\text{–}15\end{matrix}\right\}$ (16038) to 13–18–10 (16788) *36 days*. Normal adrenal tissue. There are cells containing yellow pigment; and fat cells are also present. In places, cholesterin crystals are surrounded by foreign body giant cells. There is fibrillar connective tissue in the periphery. No lymphocytic infiltration. Grade 6.

4. Two thyroids from 13–18–10 (16787) and one spleen from 13–21–11 (21161) to 13–21–19 (20897) *27 days*. Thyroids and parathyroids closely resemble autotransplants, showing very little lymphocytic infiltration in the center. Spleen contains Malpighian bodies with large endothelial cells in their centers. There are mitoses in and around lymph follicles; pulp with blood vessels and some connective tissue; mitoses in the endothelium of the sinuses; hemorrhage, and in the periphery of the transplant, collection of lymphocytes. Grade 5.25.

These experiments prove again that in family 13 a complete homogeneity of individuality differentials has not yet been attained. As in our previous experiments the individuality differentials resemble each other more on the average than the individuality differentials of brothers in non-inbred families. We find that spleen, adrenal, and liver tissue can be successfully transplanted in such

inbred families. Especially in the liver transplant, mitoses are found in bile ducts as well as in liver cells as late as 36 days after transplantation.

SERIES F. *Multiple simultaneous transplantations in the same family, examination taking place more than four months after transplantation.*

1. Thyroid, cartilage, bone, liver and adrenal from 32–20–9 (23396) to 32–20–7 (23588) *129 days*. Thyroid, parathyroid, cartilage, bone marrow with megalokaryocytes preserved; bone and proliferating zone of cartilage near bone well preserved. Liver not definitely found. Grade 6.

2. Thyroid, cartilage, liver and adrenal from 13–23–3 (23525) to 13–21–13 (23448) *132 days*. Thyroid, parathyroid, cartilage, bone, with zone of proliferating cartilage cells, bone marrow, with myelocytes, leucocytes, megalokaryocytes, well preserved; perhaps some slight connective tissue increase in bone marrow. No adrenal or liver found. Grade 6.

3. Thyroid, cartilage, adrenal, and liver from guinea-pig 13–23–5 (23525) to 13–21–3 (23660) *132 days*. Thyroid: in center a considerable mass of lymphocytes. Some lymphocytes penetrate between acini. Also in fat tissue, a mass of lymphocytes. Connective tissue in center and elsewhere increased. Good ring of acini with good colloid. Cartilage resembles autotransplant; bone marrow well preserved. No definite liver or adrenal tissue found. Grade 4.75?

4. Thyroid, cartilage and adrenal from 32–20–9 (23396) to 32–19–6 (23601) *129 days*. Thyroid: lymphocytic infiltration in center and fat tissue, which extends also between acini: parathyroid markedly infiltrated and destroyed by lymphocytes. Around cartilage perhaps very slight increase in fibrous tissue and a few lymphocytes. Proliferation zone and cartilage cells near bone preserved. Bone marrow preserved, (megalokaryocytes, leucocytes) but much lymphocytic infiltration and connective tissue increase in peripheral parts. No adrenal found. Grade 5.

5. Thyroid, adrenal and liver from 2–17–30 (23458) to 2–17–18 (23551) *132 days*. Thyroid: acini close together with colloid; in addition numerous other acini without colloid and some of these compressed. Over large areas very small acini with colloid staining red with eosin. The acinus cells of the small acini may be destroyed. In many places the thyroid appears still like an autotransplant; but

an intense lymphocytic infiltration is beginning in other places, the appearance is that of a lymph gland and the thyroid is being destroyed. The lymphocytes infiltrate also the interstices between acini and later enter the acini. Apparently some remnants of liver in the form of yellow tissue; much fibrous tissue formation and dense masses of lymphocytes. Lymph vessels filled with lymphocytes. Grade 4. This case illustrates the secondary late infiltration and destruction of syngenesiotransplants by lymphocytes.

6. Thyroid, cartilage, adrenal and liver from 2–17–30 (23458) to 2–19–13 (23347) *132 days*. No thyroid or liver found. Cartilage well preserved, but some lymphocytic collections around cartilage and in fat tissue; they are larger than they would be in autotransplants. Well preserved muscle tissue, probably transplanted. Tissue consisting of yellow vacuolar cells with nuclei, surrounded by fibrous tissue with lymphocytic masses. Tissue largely infiltrated and destroyed by lymphocytes. (Adrenal?) Grade 4.75?

7. Cartilage, ovary, liver, adrenal from 32–23–2 (23420) to 32–18–7 (21067) *129 days*. Well preserved cartilage and bone, surrounded by fat tissue. No increase in connective tissue, no lymphocytes. Proliferating zone of cartilage cells near bone. Bone marrow preserved. Cartilage partly vacuolar and dissolved; no lymphocytes. Ovary; preserved follicles of all sizes and normal ova, follicles in stage of granular degeneration, some atretic follicles. Germ layer: fibrillar connective tissue and muscle tissue well preserved. Medullary ducts; almost normal ovary. Liver and adrenal not found. Grade 6.

8. Cartilage, ovary, spleen from 2N–31 (19998) to 2–17–23 (23428) *132 days*. Cartilage well preserved surrounded by fibrillar connective tissue. Distinct lymphocytic infiltration around cartilage. Over wide areas no lymphocytic infiltration. Two ovaries with much fibrous tissue and dense lymphocytic infiltration; also fimbria epithelium with lymphocytic infiltration. Atretic follicles with zonae pellucidae. One of the two ovaries has no good follicles, the other has primordial follicles with normal ova, small follicles partly without ova, and medullary ducts, the epithelium of which has been penetrated by lymphocytes. Spleen consists of fibrous tissue with hemorrhage. Connective tissue grows into transplant. Apparently some reticulo-endothelial tissue left. There is lymphocytic infiltration. It is difficult to determine how much of these lymphocytic

masses are Malpighian bodies of the transplanted spleen and how much represents lymphocytic infiltration on the part of the host. Grade 4? It is interesting to compare experiments 7 and 8. In experiment 7, the transplants behaved like autotransplants and the ovaries are in excellent condition. In experiment 8, there is decided syngenesio-reaction with lymphocytic infiltration, affecting also the ovaries; here the condition of the ovaries is much inferior.

9. Cartilage, spleen, testicle, pancreas from 13–22–11 (23543) to 13–21–13 (20808) *132 days*. Cartilage normal, surrounded by fat tissue; in places small collections of lymphocytes around vessels. Very small collections of lymphocytes next to perichondrium. Testicle; tubules surrounded by much lymphocytic infiltration; no spermatozoa. Sertoli cells preserved. Spleen; sinuses separated by connective tissue; lymph follicles with large endothelial cells, some showing mitoses. Hemorrhagic areas into which connective tissue grows. Several serous cysts with much lymphocytic infiltration. Grade 5.

In three of these nine experiments the grade was 6; namely in families 32 in two cases, in family 13 in one case. In six experiments the results corresponded to syngenesiotransplantation. Grade 5, in family 32 and 13. Grade 4.75 in family 2 and 13. Grade 4 in two transplantations in family 2.

In families 32, 2 and 13 there was therefore non-identity of individuality differentials. The correspondence in the behavior of different organs in the same experiment is of interest; our previous results are thus confirmed. Of interest also is the late invasion of these transplants by lymphocytes. The invasion can become overwhelming and in the end destroy the transplant. Thyroid, cartilage, bone, bone marrow, parathyroid and ovary can relatively easily be transplanted; somewhat less readily spleen. Liver and adrenals were usually not found four months or later following transplantation. While thus individuality differentials are not yet identical in these families, they have reached a stage where they have become very similar to each other within the same family.

II. Homoiotransplantations as Control Experiments

Three kinds of control experiments were carried out.

A. *Transplantations from one inbred family to another inbred family.*

B. *Transplantations in control stock B.* These are guinea-pigs which originally are from the same stock from which the inbred families were derived, but in this B stock, matings as close as those between second cousins have been avoided.

C. *Transplantations from B stock to a totally unrelated stock obtained in St. Louis.* It will be possible to report on those control experiments very briefly because they behave like typical homoiotransplantations, on which one of us has reported previously on several occasions.

Series A. *Exchange of tissue between two inbred families.*

1. From family 32 to family 2. *20 days.* Thyroid in part preserved, in part infiltrated and destroyed by connective tissue and lymphocytes. In dense fibrous tissue much lymphocytic infiltration. Also parathyroid infiltrated by lymphocytes. Cartilage: part of fat tissue replaced by fibrous tissue; lymphocytic infiltration in fat tissue and around cartilage. Grade 3.25.

2. From family 32 to family 13. *20 days.* Thyroid: only fibrous tissue. Cartilage: partly necrotic; surrounded by fibrous tissue. Lymphocytes around cartilage. Bone marrow replaced by fibrillar connective tissue. Grade 1.

3. From family 32 to family 2. *20 days.* Thyroid: intense lymphocytic infiltration, some small areas of thyroid preserved. Cartilage with some perichondrial regeneration. Intense lymphocytic infiltration and connective tissue around cartilage; only slight amounts of fat tissue preserved. Grade 2.5.

4. From family 32 to family 35. *21 days.* Thyroid: some compressed acini without colloid, a few acini with colloid. Fibrous tissue and much lymphocytic infiltration between acini. Fibrous tissue around cartilage; fat tissue with thickened septa. Distinct lymphocytic infiltration. Grade 2.75.

5. From family 32 to family 13. *21 days.* Thyroid not found. Cartilage with lymphocytic infiltration. Bone marrow transformed into myxoid connective tissue. Grade 1.5.

6. From hybrid 35 plus 32 to hybrid 39 plus 13. *35 days.* No thyroid left. Cartilage surrounded by dense fibrous tissue; marked lymphocytic infiltration. Proliferative zone of cartilage near bone necrotic. Bone marrow replaced by fibrillar connective tissue and lymphocytes; capillaries with connective tissue penetrating into bone. Grade 1.

Average grade in this series is 2.

SERIES B. *Transplantation in control stock B.*

1. B–16854 to B 16870. *24 days.* No thyroid (fibrous tissue only); cartilage mostly surrounded by fibrillar connective tissue, with much lymphocytic infiltration. Grade 1.

2. B–16870 to B–16854. *24 days.* Grade 1.

3. B–16854 to B–16870. *24 days.* Grade 1.

4. B–16870 to B–16854. *24 days.* Grade 1.

5. B–16853 to B–16872. *25 days.* Thyroid: around necrotic connective tissue some acini surrounded by fibrous tissue with no, or very little, colloid. Lymphocytes infiltrate acini. Cartilage surrounded by fibrillar connective tissue and areolar tissue with fibrous septa. Lymphocytic mantle around cartilage. Grade 2.

6. B–15681 to B–15536. *25 days.* Thyroid very small. In fibrous tissue, infiltrated with lymphocytes, are small lumina of acini with remnants of colloid. Epithelium hardly recognizable. Fibrillar connective tissue with lymphocytes surround these acini, which are in process of destruction; many have already been destroyed. Hyaline fibrous tissue in center. Cartilage preserved, surrounded by fibrous tissue with variable amounts of lymphocytes. Remnants of areolar tissue. Lymphocytes penetrate a little into peripheral cartilage. Grade 2.25.

Average grade, 1.37.

SERIES C. *Transplantation from B stock to unrelated St. Louis stock.*

1. B to Bu. *40 days.* Compressed remnants of thyroid; lymphocytic infiltration. Fibrous tissue around cartilage. Lymphocytes penetrate into periphery of cartilage. Grade 2.

2. B to Po. *20 days.* Lymphocytes penetrate into periphery of perichondrium. Grade 1.

3. B to Po. *30 days.* No thyroid. Lymphocytes penetrate in places into cartilage. Grade 1.25.

4. B to Bu. *40 days*. Grade 1.

Average grade 1.31.

Comment. The differences between these three series of homoiotransplantation and the six series in which transplantations were carried out in the same family are very striking; in the former the total average grade is 1.6; in the latter series the average grade is 5.28. Of course, these figures can only claim to be approximate. We may state that the average grade in the transplantations within the same family is between 5 and 6, but nearer 5 than 6. There is an indication that families 32 and 13 are more strange to each other than families 32 and 2 or 32 and 35. However, this point needs further investigation. Of interest is also the marked reaction against tissues of hybrids in homoiotransplantation.

III. Transplantation from Brother to Brother within an Inbred Family

When, in an ordinary non-inbred strain, transplantations from brother to brother are carried out, the results are better than in ordinary homoiotransplantation, or in syngenesiotransplantation between children and parents or *vice versa*.[1] It was of interest to determine whether in case of brother to brother transplantations within the inbred family the results would equal those of autotransplantation, and whether the individuality differentials between brothers in the inbred family had become identical.

We carried out two series of experiments in this direction; in the first one, series A, we used individuals belonging to the same family and in series B we exchanged tissues between brothers which were hybrids, the parents belonging to different inbred families.

Series A:

1. From 35–23–8 (16715) to 35–23–8 (16716) *5 months, 16 days*. Thyroid and parathyroid behave like autotransplants. Grade 6.

2. From 32–19–9 (16900) to 32–19–9 (16899) *4 months, 9 days*. Grade 6 (included among successive transplantations).

3. From 2N–26 (15673) to 2N–26 (15674) *61 days*. Grade 6. Thyroid with auto-structure, acini close together. Solid retracted colloid, medium-sized epithelium. In one place there is a small number of lymphocytes. Cartilage well preserved, surrounded by areolar and fat tissue. No lymphocytes.

4. From Y–4$\left\{\begin{matrix}13–20–2\\13–19–5\end{matrix}\right\}$(16039) to Y–4$\left\{\begin{matrix}13–21–2\\13–19–5\end{matrix}\right\}$(16038) *40 days*. Transplant with striated muscle well preserved. Grade 6. (The corresponding transplantation to a relative a little further removed produced a syngenesio-reaction.)

5. Thyroid; cartilage and salivary gland from 2N–20 (15618) to 2N–20 (15619) *39 days*. Grade 6.

6. From 2–18–4 (15693) to 2–18–4 (15692) *49 days*. No connective tissue, new formation, nor lymphocytes. Grade 6.

7. From 13–19–9 (15649) to 13–19–9 (15648) *35 days*. Thyroid with auto-structure, but in places in center and between acini around vessels are masses of lymphocytes surrounding and destroying acini. In a corresponding transplant in more distantly related animals of the same kind, there is much more lymphocytic infiltration than in brother to brother transplantation. Cartilage partly necrotic. Regenerating perichondrial cartilage infiltrates and replaces the necrotic cartilage. Connective tissue grows as papillae into necrotic cartilage, and the individual connective tissue cells grow also singly into it. Bone seems to have been produced in places where there was necrotic cartilage. In areolar tissue around cartilage some increase in connective tissue. Some megalokaryocytes, new bone marrow. Grade 5.

8. From 32–17–10 (15611) to 32–17–10 (15609) *40 days*. Grade 6.

9. From 32–17–11 (15575) to 32–17–11 (15574) *10 days* (died). Grade 6.

10. From 2–17–10 (15959) to 2–17–10 (15960) *50 days*. Grade 6.

11. From 2–17–5 (15695) to 2–17–5 (15696) *30 days*. Grade 6.

12. From 2–17–5 (15695) to 2–17–5 (15694) *30 days*. Grade 6.

13. From 2N–20 (15617) to 2N–20 (15616) *12 days* (died). Thyroid: very little lymphocytic reaction, but in places around the vessels there are small collections of lymphocytes. Cartilage similar to autotransplant. Grade 5.25.

14. From 2N–20 (15617) to 2N–20 (15619) *45 days*. Grade 6.

Comment. In these 14 experiments the examinations were made after *five months, sixteen days; four months, nine days; 61, 50, 49, 45, 40, 40, 39, 35, 30, 30, 12* and *10 days*. In twelve cases the grade was 6, which means that the pieces showed the character of autotransplants; the individuality differentials between brothers in the same inbred family were identical as far as this test indicates. But this

statement is correct only with the reservation that possibly a reaction might still have occurred, if the pieces had been allowed to stay in the host for a still longer period of time. Therefore, we can only say definitely that in these twelve cases the individuality differentials of brothers closely approached or perhaps reached the condition found in autotransplant. The families in these twelve cases were: 35, one case; 32, three cases; 2, six cases; 13, two cases. In one case in family 13 there was a distinct syngenesio-reaction (Grade 5) and in one case in family 2 there was probably a very mild syngenesio-reaction (Grade 5.25). It is again family 13 in which individuality differentials are so distinctly divergent that even against the tissues of brothers a reaction is elicited; probably also in family 2N identity between the individuality differentials of brothers has not yet been entirely reached. The latter result is in accordance with the mixed breeding (within family 2) of mating 2N–20. The average grade in this series was 5.87 or 5.92 if experiment 13 is omitted.

SERIES B. 1. C–1–58 $\left\{\begin{matrix}13\text{–}13\text{–}16\\32\text{–}14\text{–}13\end{matrix}\right\}$ (16050) to C–1–58 $\left\{\begin{matrix}13\text{–}13\text{–}16\\32\text{–}14\text{–}13\end{matrix}\right\}$ (16052)

40 days. Two transplanted lobes of thyroid with structure of autotransplants. Well preserved parathyroid. Marked lymphocytic infiltration in center of thyroid and in parathyroid, and some infiltration between acini. Lymphocytes have infiltrated and destroyed a great part of areolar tissue. Cartilage and striated muscle tissue well preserved, surrounded by good areolar tissue; connective tissue penetrates into necrotic cartilage; around vessels and cartilage in places a slight mantle of lymphocytes. Grade 5. We have here to deal with the F2 generation of hybrids between families 13 and 32; this fact may explain the lack of auto-reaction in this case.

2. C–O–237 $\left\{\begin{matrix}39\text{–}14\text{–}10\\2\text{–}13\text{–}7\end{matrix}\right\}$ (15891) to C–O–237 $\left\{\begin{matrix}39\text{–}14\text{–}10\\2\text{–}13\text{–}7\end{matrix}\right\}$ (15893)

35 days. Thyroid and cartilage about like autotransplants. Very slight lymphocytic collections in center which are probably still within the range of that found in autotransplants. Grade about 6.

3. C–O–237 $\left\{\begin{matrix}39\text{–}14\text{–}10\\2\text{–}13\text{–}7\end{matrix}\right\}$ (15891) to C–O–237 $\left\{\begin{matrix}39\text{–}14\text{–}10\\2\text{–}13\text{–}7\end{matrix}\right\}$ (15894)

35 days. No lymphocytes. No increase in connective tissue. Grade 6.

4. C–O–268$\left\{\begin{matrix}32\text{–}16\text{–}9\\39\text{–}14\text{–}17\end{matrix}\right\}$(16023) to C–O–268$\left\{\begin{matrix}32\text{–}16\text{–}9\\39\text{–}14\text{–}17\end{matrix}\right\}$(16025)

35 days. Thyroid and cartilage. Grade 6.

5. C–O–252$\left\{\begin{matrix}13\text{–}14\text{–}13\\32\text{–}15\text{–}14\end{matrix}\right\}$(16795) to C–O–252$\left\{\begin{matrix}13\text{–}14\text{–}13\\32\text{–}15\text{–}14\end{matrix}\right\}$(16794)

25 days. Thyroid approaching autotransplant; some slight diffuse lymphocytic infiltration in center; also in small peripheral area some lymphocytes. In places around cartilage a slight amount of newly formed connective tissue. Grade 5.5.

6. Reciprocal to (5): 16794 to 16795. Thyroid and cartilage, *25 days*. Grade 6.

7. C–O–234$\left\{\begin{matrix}2\text{–}13\text{–}7\\35\text{–}16\text{–}20\end{matrix}\right\}$(17078) to C–O–234$\left\{\begin{matrix}2\text{–}13\text{–}7\\35\text{–}16\text{–}20\end{matrix}\right\}$(17079)

35 days. Thyroid like autoptranslant. A small number of lymphocytes around vessels in center, probably within the range of that found in autotransplantation. Cartilage like autotransplant. Grade 6.

8. C–O–285$\left\{\begin{matrix}35\text{–}21\text{–}2\\32\text{–}15\text{–}15\end{matrix}\right\}$(16756) to C–O–285$\left\{\begin{matrix}35\text{–}21\text{–}2\\35\text{–}15\text{–}15\end{matrix}\right\}$(16757)

26 days. Thyroid like autotransplant; cartilage perhaps with slightly increased connective tissue and with some lymphocytes. Grade 5.75?

Comment. In these eight experiments the time of examination varied between 40 and 25 days. In five experiments the grade was 6. The hybrids in these cases were combinations of 39 plus 2; 39 plus 32; 13 plus 32; 2 plus 35. In three cases the transplants were not identical with autotransplants. The combinations and grades in these cases were as follows: 13 plus 32, grade 5; 13 plus 32, grade5.5; 35 plus 32, grade 5.75. In the case of the last of these hybrids, experiment 8, it is doubtful whether grade 6 has not been reached. In experiments 5 and 6 (hybrid 13 plus 32) reciprocal transplants did not give exactly the same result, although the reactions in both cases were similar. It is again those hybrids, into whose composition family 13 entered, that do not yet reach the identity of individuality or 5.91 if we exclude experiments III A–13 and III B–1 differentials in brothers. The average grade in this series is 5.78, 5.89 if we omit experiment III B–1, or 5.91 if we exclude experiments III A–13 and III B–1. The average grade in series A and B combined is 5.84. These average grades also indicate that a perfect identity of the individuality differentials has not yet been reached

in all cases, although on the whole they closely resemble one another. The figure for the average is here higher than in the case of the exchange of tissues in the same family between individuals which belonged to the same inbred family, but were not nearly related. In the latter case the average grade was 5.28.

IV. Syngenesiotransplantations (Brother to Brother) in Non-Inbred Families

We have previously analyzed syngenesiotransplantation in non-inbred families of guinea-pigs. We found as the average grade of brother to brother transplantation 3.6. We shall here briefly state the result of brother to brother transplantation in the B strain of guinea-pigs for comparison with the results obtained in brother to brother transplantation within inbred families.

There were fifteen experiments; examination took place 25 to 40 days following transplantation. Grades: 6; 5.5; 5; 5; 5; 4.75; 3.75; 3.25; 3; 2; 2; 2; 1; 1; 1. Average grade 3.35.

A few examples may suffice. 1 B–202 to B–202, *35 days*. No thyroid found. Fibrous tissue surrounds cartilage which is partly cellular. Marked lymphocytic mantle around cartilage, but in places loose connective tissue with fewer lymphocytes. The latter penetrate into cellular cartilage. Epithelioid reaction in fat tissue.

2. B–274 to B–274. *40 days*. Thyroid very well preserved; good acini, close together with good solid restricted colloid. A little connective tissue in center; also a little areolar tissue with a small number of lymphocytes in one place in center. Cartilage well preserved surrounded by areolar tissue; no lymphocytes. Grade 6.

3. B–261 to B–261. *35 days*. Thyroid. Remnants of acini, some containing colloid, surrounded by a mass of lymphocytes. Apparently remnants of parathyroid infiltrated by lymphocytes; especially in center of parathyroid much lymphocytic infiltration. Periphery of ring of acini surrounded by lymphocytes. Well preserved cartilage; where it is thicker, it is partly shrunken. Cellular cartilage is surrounded by areolar tissue which includes strands of connective tissue and lymphocytes, especially near the perichondrium and around vessels. However, the greater part of areolar tissue contains no lymphocytes. Grade 3.25.

Comment. The average grade for the syngenesiotransplants (brother to brother transplantations in non-inbred families) is con-

siderably lower than the average grade of ordinary homoiotransplantations in the inbred families and still lower than the grade for the brother to brother transplantations in the latter.

V. Transplantation from Hybrid to Pure Component Family

In this series a male belonging to one of the inbred families and a female belonging to a different family were mated, and the hybrid thus obtained was used for transplantation to another individual who was not related to the hybrid but belonged either to the family of his father or mother. Thus twenty-four experiments were carried out. The length of time during which the pieces were left in the host varied between 37 and 20 days. In one additional case the host died 15 days after transplantation.

The relationships between host and donor will be brought out more distinctly in the following list of experiments:

1. C–O–282 $\left\{\begin{matrix} 2\text{–}15\text{–}3 \\ 32\text{–}17\text{–}7 \end{matrix}\right\}$ (15566) to (32–17–11 (15575) *24 days.*

Grade 4.25. Thyroid: structure of autotransplant, but dense lymphocytic masses in center and in places in peripheral fibrous capsule around vessels. Lymphocytes penetrate from center towards periphery between acini. Many acini have been destroyed. Some connective tissue increase in center and around acini. Cartilage: well preserved surrounded by areolar tissue with slight increase in connective tissue and lymphocytes.

2. Same to 2N–20 (15618) *24 days.* Grade 4.

3. C–O–298 $\left\{\begin{matrix} 13\text{–}21\text{–}4 \\ 2\text{–}16\text{–}17 \end{matrix}\right\}$ (18188a) to 13–20–13 (15784) *35 days.* Grade 2.

4. Same to 2–16–17 (18059) *35 days.* Grade 1.

Thyroid: only fibrous tissue found. Cartilage: with a great deal of necrosis; some shrunken cells. Much connective tissue and moderate mantle of lymphocytes around cartilage, the necrotic parts of which are entered by some connective tissue and lymphocytes.

5. C–O–304 $\left\{\begin{matrix} 32\text{–}19\text{–}10 \\ 2\text{–}15\text{–}16 \end{matrix}\right\}$ (18305) to 32–18–15 (16753) *37 days.* Grade 3.75.

6. Same to 2N–24 (18043) *37 days.* Grade 3.25.

Thyroid: very good acini, close together in places and with solid retracted colloid. Much lymphocytic infiltration around and between acini. Also in center of thyroid lymphocytes surround some

acini and penetrate into thyroid in places; lymph vessels filled with lymphocytes. The greater part of the thyroid is intact, but in the connective tissue around acini there is much lymphocytic infiltration. Lymphocytes penetrate also into colloid. There are areas of fibrous tissue, of areolar tissue and of lymphocytic infiltration around cartilage. Striated muscle tissue preserved at one end.

7. C–O–240 { 32–16–9 / 35–16–11 } (15943) to 32–19–9 (16901) *37 days.* Grade 4.

8. Same to 35–23–8 (16716) *37 days.* Grade 2.

9. C–O–297 { 2–17–8 / 35–23–10 } (18063) to 35–12 (18589) *25 days.* Grade 2.50.

10. Same to 2N–25 (18184) *25 days.* Grade 3.

11. C–O–282 { 2–15–3 / 32–17–7 } (15567) to 32–19–8 (15633) *25 days.* Grade 6.

12. Same to 2–17–5 (15696) *25 days.* Grade 3.

13. C–O–237 { 39–14–10 / 2–13–7 } (15893) to 39–16–21 (15727) *25 days.* Grade 2.5.

14. Same to 2–18–4 (15692) *25 days.* Grade 2.85.

15. C–O–297 { 2–17–8 / 35–23–10 } (18066) to 2N–31 (19998) *20 days.* Grade 2.75.

Much thyroid tissue destroyed, relatively little thyroid left. The remaining ring of acini with much lymphocytic infiltration and much fibrous tissue formation. Around cartilage still some muscle tissue preserved.

16. Same to 35–22–17 (17966) died after *15 days.* Grade 3.25. (Some muscle tissue preserved.)

17. C–O–298 { 13–21–4 / 2–16–17 } (18190) to 13–21–8 (18185) *21 days.* Grade 1.25. Thyroid destroyed; merely dense fibrous tissue and some lymphocytes surrounded by fat tissue and epithelioid and giant cells. Bone marrow replaced by loose connective tissue. Cartilage mostly necrotic. Some fat tissue mingled with epithelioid and giant cells. Over wide areas cartilage surrounded by connective tissue with some lymphocytic infiltration. Near bone, a zone of cartilage is living.

18. Same to 2N–32 (20338) *21 days.* Grade 2.

19. C–O–269 { 32–16–9 / 39–14–17 } (16025) to 32–19–8 (15633) *23 days.* Grade 5.5. Large thyroid with solid retracted colloid; medium-sized

epithelium. Many large acini. A small amount of connective tissue in center. No lymphocytes except in one connective tissue septum, where there is a slight collection. Well preserved parathyroid. Cartilage well preserved, surrounded by areolar tissue. There is a slight increase in connective tissue with very little lymphocytic infiltration in areolar and fat tissue.

20. Same to 39–16–21 (15633) *23 days*. Grade 1.25.

21. C–O–304 $\left\{\begin{matrix}32\text{–}19\text{–}10\\2\text{–}15\text{–}16\end{matrix}\right\}$ (18306) to 2N–25 (18182) *29 days*. Grade 5.5.

22. C–O–284 $\left\{\begin{matrix}39\text{–}13\text{–}13\\32\text{–}17\text{–}5\end{matrix}\right\}$ ( ) to 39–16–21 (15727) *25 days*. Grade 3.25.

23. Same to 32–17–11 (15574) *25 days*. Grade 5.

24. C–O–297 $\left\{\begin{matrix}2\text{–}17\text{–}8\\35\text{–}23\text{–}10\end{matrix}\right\}$ (17093) to 2–17–5 (15696) *31 days*. Grade 4.

The hybrid combinations used were as follows: $\frac{2}{32}$ to 2 in four different experiments; $\frac{2}{32}$ to 32 in three different experiments.

(1) $\frac{2}{32}$ to 32, *24 days*. Grade 4.25. (2) $\frac{2}{32}$ to 2N, *24 days*. Grade 4. (5) $\frac{32}{2}$ to 32, *37 days*. Grade 3.75. (6) $\frac{32}{2}$ to 2N, *37 days*. Grade 3.25. (11) $\frac{2}{32}$ to 32, *25 days*. Grade 6. (18) $\frac{2}{32}$ to 2. *25 days*. Grade 3. (21) $\frac{32}{2}$ to 2N, *29 days*. Grade about 5.5.

$\frac{13}{2}$ was used in four experiments. (3) $\frac{13}{2}$ to 13, *35 days*. Grade 2. (4) $\frac{13}{2}$ to 2, *35 days*. Grade 1. (16) $\frac{13}{2}$ to 13, *21 days*. Grade 1.5. (17) $\frac{13}{2}$ to 2N, *21 days*. Grade 2. In transplantations in which family 13 enters as a component of a hybrid, the reactions against the transplants are more severe than in transplantations in which family 32 takes the place of family 13.

$\frac{32}{35}$ was used in two experiments. (7) $\frac{32}{35}$ to 32, *37 days*. Grade 4. (8) $\frac{32}{35}$ to 35, *37 days*. Grade 2.

$\frac{2}{35}$ was used in five experiments. (9) $\frac{2}{35}$ to 35, *25 days*. Grade 2.50. (10) $\frac{2}{35}$ to 2N, Grade 2. (14) $\frac{2}{35}$ to 2N, *20 days*. Grade 2.75. (15) $\frac{2}{35}$ to 35 (died after *15 days*). Grade 3.25. (24) $\frac{2}{35}$ to 2, *31 days*. Grade 4. In these experiments the results were almost but not quite as unfavorable as in transplantation for hybrid $\frac{13}{2}$.

$\frac{39}{2}$ was used in two experiments. (12) $\frac{39}{2}$ to 39, *25 days*. Grade 2.5? (13) $\frac{39}{2}$ to 2, *25 days*. Grade 2.75–3. In this combination the results were likewise unfavorable.

Hybrid $\frac{32}{39}$ was used in four experiments. (19) $\frac{32}{39}$ to 32, *23 days*. Grade 5.5. (20) $\frac{32}{39}$ to 39, *23 days*. Grade 1.25. (22) $\frac{39}{32}$ to 39, *25 days*. Grade 3.25. $\frac{39}{32}$ to 32, *25 days*. Grade 5. In this combination the results were relatively favorable.

In three experiments, the result approaches that in autotransplantation; in one experiment the result corresponded to a favorable syngenesiotransplantation. In nine cases the result was a very pronounced syngenesio-reaction. In three cases the result was on the border-line between a syngenesio- and homoio-reaction and in eight experiments a homoio-reaction was obtained. The average grade was 3.25. The average grade was therefore only very slightly less favorable than that obtained in brother to brother transplantation in non-inbred families.

We see thus that the grades in the different experiments vary as much as between 6 and 1. The grades were as follows: 6; 5.5; 5.5; 5; 4.25; 4; 4; 4; 3.75; 3.25; 3.25; 3.25; 3; 3; 2.85; 2.75; 2.50; 2.50; 2; 2; 2; 1.5; 1.25; 1.

In eleven experiments we transplanted the organs of one hybrid to both the component parent strains; in none was the transplantation made to the direct parents of the hybrids. In seven of these experiments the results were similar after transplantation into both parent strains, in three experiments they were very different, and in

one case the difference, although noticeable, was not quite so pronounced. Different results were obtained in the following combinations: $\frac{2}{32}$ to 32, grade 6. $\frac{2}{32}$ to 2 grade 3. $\frac{32}{35}$ to 32, grade 4. $\frac{32}{35}$ to 35, grade 2. $\frac{32}{39}$ to 32, grade 5.5. $\frac{32}{39}$ to 39, grade 1.25. $\frac{39}{32}$ to 39, grade 3.25. $\frac{39}{32}$ to 32, grade 5.

In general it seems that hybridizations, into which family 32 enters as one of the strains, are relatively favorable; the reactions on the whole are slight, at least in a number of these cases, and especially when the host belongs to family 32. The presence of a single dose of 2, 35 or 39 family-differential does not necessarily lead to severe reactions if a guinea-pig belonging to family 32 is the host, but in other cases it may do so. If a member of family 2 is host, the reactions against hybrid tissue $\frac{32}{2}$ are usually more marked than the reactions on the part of a 32 host, but here also they may be very slight. Similarly host 32 reacts much less actively against $\frac{32}{35}$ hybrid tissue than a 35 host. In host 35 the reactions are pronounced against hybrid tissue of 35 with 2 or 32. Host 32 showed relatively slight reactions against $\frac{32}{39}$ hybrid tissue, while host 39 showed decidedly more marked reactions against the same tissue. Host 2 showed quite noticeable reactions against $\frac{2}{35}$ and $\frac{2}{39}$ hybrid tissues. Among the most marked reactions were those obtained against hybrid tissue in which 13 entered as a component. The reactions occurred in host 2 as well as in host 13. Throughout our experiments we found the most marked reaction within family 13, which represents probably the least homozygous of the various families. Not only is the reaction against family 13 most marked on the part of other families, but family 13 as host reacts likewise most actively against the individuality differential of the other families. We may assume that the different families show a varying degree of relationship to each other, and that while the strange genes in general cause a reaction on the part of the host, perhaps certain genes call forth a more severe reaction than others, and furthermore that certain

families are possibly less sensitive to differences in genes than other families. The experiments recorded so far in these series suggest these conclusions.

VI. Transplantation from Pure Component Family to Hybrid

In this series the reverse transplantations were carried out. Tissues were transferred to a hybrid from an individual belonging to one of the two inbred families, members of which had been used for hybridization. Host and donor were not directly related to each other, except in so far as all members of an inbred family are in certain respects so related.

Fourteen experiments were carried out in this series; the period during which the transplants remained in the host varied in the different cases between 25 and 35 days.

The following experiments were made:

1. 2N–25 (18184) to C–O–297 $\left\{\begin{matrix} 2\text{–}17\text{–}8 \\ 35\text{–}23\text{–}10 \end{matrix}\right\}$ (18063) *25 days.* Grade 6. Thyroid: with auto-structure; acinus cells of low to medium height, solid retracted colloid. Some loose connective tissue and large vessels in center. No lymphocytes, no increase in connective tissue. Cartilage well preserved; normal fine fibrillar connective tissue and fat tissue surround the cartilage. Again no lymphocytes; no increase in connective tissue.

2. 351–2 (18589) to same hybrid, *27 days.* Grade 5.25. Thyroid, as in preceding experiment, but some masses of lymphocytes penetrate from outside to center. Cartilage also well preserved and surrounded by fat tissue, but in places some fibrillar connective tissue with a few lymphocytes around it. In this case a very slight reaction took place.

3. 2–16–17 (18059) to C–O–298 $\left\{\begin{matrix} 13\text{–}21\text{–}4 \\ 2\text{–}16\text{–}17 \end{matrix}\right\}$ (18188a) *35 days.* Grade 6.

4. 13–21–13 (15784) to same hybrid. Grade 6.

5. 32–18–15 (16753) to C–O–304 $\left\{\begin{matrix} 32\text{–}19\text{–}10 \\ 2\text{–}15\text{–}16 \end{matrix}\right\}$ (18305) *35 days.* Grade 6. Only a small collection of lymphocytes around foreign body; otherwise like autotransplant.

6. 2N–24 (18043) to same hybrid. *35 days.* Grade 6. As in the majority of other transplants, the perichondrium produces in places

new cartilage, which either penetrates adjoining necrotic cartilage and replaces it or which is deposited in the form of a plate.

7. 32–19–9 (16900) to C–O–240 $\left\{\begin{matrix}32–16–9\\35–16–11\end{matrix}\right\}$ (15943) *35 days.* Grade 6. Transplants resemble autotransplants. Into peripheral necrotic cartilage some connective tissue is growing and in places it penetrates superficially even adjoining living cartilage. A very few small strands of lymphocytes in fat tissue; conditions are probably still within the range of those observed in autotransplants.

8. 35–23–8 (16716) to same hybrid. *35 days.* Grade 5.75. Thyroid and parathyroid like autotransplants, except that we find in the parathyroid in one place a slight, but distinct collection of lymphocytes which exceeds in size collections found in cases of autotransplantation. In areolar and fat tissue around cartilage there are few polymorphonuclear leucocytes and lymphocytes. There may also possibly be here and there a very slight increase in connective tissue.

9. 32–18–9 (18179) to C–O–304 $\left\{\begin{matrix}32–19–10\\2–15–16\end{matrix}\right\}$ (18306) *25 days.* Grade 6.

10. 2N–25 to same hybrid *25 days.* Grade 6. Thyroid transplant consists of ring of well preserved acini with a small amount of connective tissue and areolar tissue in center. Colloid in acini is solid and somewhat retracted. Cartilage is surrounded by areolar and fat tissue.

11. 13–18–10 (16788) to C–O–298 $\left\{\begin{matrix}13–21–4\\2–16–17\end{matrix}\right\}$ (18190) *32 days.* Grade 5.50. Thyroid, parathyroid and cartilage like autotransplants, except for a collection of lymphocytes in periphery of parathyroid which also penetrates into this gland. Cartilage is well preserved, but parts are necrotic; the latter are surrounded by regenerated perichondrial cartilage which as usual consists of small cartilage cells with nuclei that are more prominent than in older cells, its blue stain contrasting within the light stain of the cytoplasm. In other places the host connective tissue grows into necrotic cartilage and replaces it. Some lymphatics in fat tissue are filled with lymphocytes.

12. 2N–25 (18182) to same hybrid. *32 days.* Grade 5.50. Thyroid like autotransplant except that in one place in the center there is a small mass of lymphocytes which probably exceeds in size the

range of lymphocytic collections found in autotransplants. In the cartilage, some regenerated transplanted muscle tissue with chains of nuclei. There is a very slight increase of connective tissue with some lymphocytes found in the areolar and fat tissue around cartilage.

13. 2N–20 (15619) to C–O–297 $\left\{\begin{matrix} 2\text{–}17\text{–}8 \\ 35\text{–}23\text{–}10 \end{matrix}\right\}$ (18066) *31 days.* Grade 5.50. Thyroid like autotransplant, except for a few small collections of lymphocytes. In cartilage transplant there is some transplanted striated muscle with only a few nuclear chains. In places there are small collections of lymphocytes around vessels in fat tissue of cartilage transplant probably exceeding the number found in autotransplants.

14. 35–22–17 (17695) to same hybrid. *31 days.* Grade 5.25. Thyroid resembles autotransplant except that there is some thickening of connective tissue septa and a slight lymphocytic infiltration. In cartilage transplant, which is well preserved, there are some distinct collections of lymphocytes in areolar and fat tissue and there is here also a very slight increase in connective tissue. Areas of necrotic cartilage are partly replaced by perichondrial cartilage which may regenerate in the form of a plate. Near the junction of xiphoid cartilage with bone, connective tissue grows into areas of necrotic cartilage and produces a bone-like substance. We find also fibrillar bone marrow with megalokaryocytes, capillaries and lymphocytes. Osteoclasts also are seen at the edge of bone marrow. Between the areas of bone, perichondrium forms cartilage. A somewhat similar condition was probably observed in experiment (8) of this series. In this transplant very well formed muscle tissue produced by transplanted muscle cells was seen.

Comment. The average grade in these fourteen experiments is 5.77, approaching therefore the condition found in autotransplantation. In eight cases the grade corresponds to the result obtained in autotransplantation. In the remaining experiments the grades are 5.75; 5.50; 5.50; 5.50; 5.25; 5.25. Grade 6 was obtained in the following transplantations: 2 to $\frac{2}{35}$; 32 to $\frac{32}{2}$ in two experiments; 2 to $\frac{32}{2}$ in two experiments; 32 to $\frac{32}{35}$; 13 to $\frac{13}{2}$ and 2 to $\frac{13}{2}$. The other grades were obtained in the following combinations: 35 to $\frac{2}{35}$ in two

experiments 2 to $\frac{2}{35}$; 13 to $\frac{13}{2}$ and 2 to $\frac{13}{2}$. Slight reactions were therefore observed in cases in which family 13 or family 35 were hosts or donors. The strongest reactions were obtained in cases in which 35 was both host and donor, and especially in experiments in which family 35, serving as host, was combined with family 2. When families 32 and 35 were combined as host and donor a reaction, although somewhat mitigated, was observed. As in the preceding series we found that hybridizations, in which families 13 and 35 were involved, gave rise to relatively stronger reactions if tissues were exchanged between hybrids and component families than if the transplantations concern families 32 and 2. In this series we have not used family 39. On the other hand, we find that exchange of tissues in cases in which families 13 and 35 enter may not necessarily give rise to reactions in all cases; the individuality differentials between donor and host may be so constituted that no reaction occurs, at least within the range of time used in the particular experiment.

These fourteen experiments may be arranged in seven groups in which an exchange of tissue took place between hybrid members of the two families in each case. In four of these groups the results were about the same without regard to the character of the donor family; in three there was some difference, in each instance the stronger reaction was obtained when family 35 furnished the donor.

If we compare the average grades in the different series, omitting those in which the number of experiments at present is still very small, we find in the transplantations within the same family an average of 5.6 or slightly higher. The multiple transplantations extending over a long period of time give a somewhat lower average, namely 5.4, as do successive transplantations, although the number of our experiments is very small in this latter subseries. Some complicating factors may enter in the case of multiple transplantations extending over a long period of time, and of successive transplantations.

In experiments in which we transplanted from one hybrid to another hybrid of the same composition, not directly related, stronger reactions seemed to be elicited, and the averages were correspondingly lower, namely grade 4; but here again the number of our experiments is as yet too small.

The average grade of 5.6 in the uncomplicated subdivision of this

series is slightly lower than the average we obtained in the last series in which we transplanted pieces from a component strain to a hybrid; here the grade was 5.77. Of course the slight difference between these figures may have no significance. The figure for the last series is about the same as that for the series in which tissues were exchanged between two brothers, which were both hybrids of the same inbred families; here the average grade is 5.78. In this case the presence of two component families in donor as well as in host is an unfavorable condition which is compensated by the fact that both host and donor are brothers. In general we have some indication that transplantations from one hybrid to another not directly related hybrid, both derived from the same families, call forth a stronger reaction and give a lower average grade (tentatively 4, a figure based however on a very small number of experiments) than transplantations within the same inbred family, if host and donor are not directly related. Transplantations from brother to brother in the inbred families are closer to autotransplantations than any of the preceding kinds of transplantations, the average grade being 5.9; this of course is in accordance with expectations. On the other hand, the transplantations from a hybrid to the component strains call forth strong reactions; the average grade is accordingly low, namely 3.25, which is very similar to the average grade in brother to brother transplantations in the B group, in which the average is 3.35.

Conclusions

From these results we may conclude that it is not the similarity or difference between individuality differentials of donor and host which determines the reaction against the transplant, but the reaction depends on the presence in the host of genetic factors of the donor. The lack in the donor of genetic factors present in the host is apparently of little or no consequence. In the case of tumor transplantations in different varieties of mice which have been inbred, although as it seems not exclusively through consecutive brother to brother matings, Little and Tyzzer[3] found that transplantations from hybrid to parent strain gave negative results as far as the number of takes was concerned, while the reverse transplantation gave 100 per cent takes. They concluded therefore that only one dose of genes is required for successful transplantation. We found in our experiments, in which we used a much finer means of measuring genetic

composition than tumor transplantations, that in different transplantations from hybrid to component strain as well as from component strain to hybrid, individual differences exist which are very pronounced in the case of transplantations from hybrid to component strain. It is probably not so much the presence of a single dose of the genes which is required, as far as the absence of reaction against the transplant is concerned, as the lack of a strange gene in the donor; while the presence of a strange gene in the host is without significance. Thus the transplantation from one hybrid to another hybrid is more injurious than transplantation from component family to hybrid.

More recently, Little and Johnson [4] found in an inbred strain of Japanese waltzing mice that transplantation of pieces of spleen from waltzing mice to hybrids of these animals with white mice, behaved like autotransplants; while spleen transplanted from such hybrids to waltzing mice behaved like very pronounced homoiotransplants, and were destroyed within a short time. In these experiments the transplantations in which the hybrids served as hosts gave much better results than the reciprocal transplantations; but again the lack of finer means of measuring genetic differences led in all probability to incorrect estimates of the genetic identity or lack of identity in the individuality differentials of host and donor. It is very probable that the results in these transplantations did not correspond exactly to those found in autotransplantation, or to those of complete homoiotransplantation, but that both were intermediate between these two extremes. However the fact brought out by Little and Johnson and also, it seems, their interpretation of these facts, appear to be in essential harmony with the conceptions which one of us had previously developed concerning the individuality differential.

The results which we obtained cannot be considered inevitable from *a priori* reasoning. Thus one might have expected that the presence in the individuality differential of the host of a set of genes which are not present in the donor and transplanted tissue should lead to essential differences between the metabolism of host and transplant and consequently to aggressive reactions on the part of the host; this evidently does not take place. It seems to be merely the presence of strange genes in the transplanted tissue which acts as a stimulus on the host and incites the cells of the latter to react.

Apparently the absence of genes in the transplant is without significance. It is the presence of strange genes in the transplanted tissue which causes the inferiority in the latter in respect to the survival and regenerative power.

Both of these consequences of transplantations of tissues, in which the individuality differentials are more or less incompatible with those of the host, agree with those established previously by one of us in his series of transplantations. The lymphocytic and connective tissue reactions, the behavior of blood vessels, the degeneration of sensitive tissues like bone marrow, liver cells and spleen tissue in accordance with incompatabilities of individuality differentials are the same in these experiments as those previously described.

As to other general conclusions, we may refer to the discussions given at the end of the various sections in this paper. We may again state that our observations point to the conclusion that in some families a homozygous condition has been reached more completely than in others, but that it has not yet been actually attained in any of these inbred families. It is probable that some families are more nearly related to each other than others and furthermore it is possible that certain strange factors present in some families and individuals cause a greater incompatibility reaction than others and, in addition, we must consider the possibility that the sensitivity and degree of reaction of certain families or individuals against individuality differentials surpass those of others.

There is some indication that in the transplantations within the same family, the individuality differentials of host and transplant are more similar to each other the larger the number of brother and sister matings which both individuals had in common before the separation of brother and sister matings in different sidelines and the smaller the number of these separate and distinct brother and sister matings for each of the two individuals in the preceding generations. Thus in family 2, three transplantations with an average grade of 4.6 were separated for twenty-four, twenty-four and twenty generations of brother and sister matings respectively; while preceding their separate matings they had six, six and nine common ancestral matings. In two other experiments in this family in which host and donor were separated for twelve and three generations only, and twelve and sixteen brother and sister matings in common, the grade was 6.

A similar result was obtained in matings in family 13. Here the average grade was 4.9 in a series of transplantations in which the number of generations of brother and sister matings since the splitting off of the sidelines of brother and sister matings varied between twenty-three and twenty-seven, while the average number of preceding brother and sister matings which both individuals had in common was slightly less than nine. In three other transplantations in which the number of generations since separation was about twelve, and the number of common generations varied between thirteen and seventeen, the average grade was 5.8. Which of these two factors, the number of generations which donor and host had in common, or the number of separate generations of brother and sister matings in these two individuals is the important one, is impossible to decide on the basis of our data. If the latter factor should play an important part it would be necessary to attribute this effect to possible mutations which occurred during the period of separate breedings: the mutations under these conditions would then have been different in the ancestry of host and donor.

In the large majority of cases in which after nineteen or twenty consecutive brother and sister matings, tissues were transplanted from brother to brother or sister the reactions taking place showed absence of incompatibility, and therefore at least apparent identity between individuality differentials of host and donor. It is therefore probable that under these conditions the difference between the individuality differentials of host and donor disappeared. These two individuals should therefore behave like identical twins. That this condition has actually been reached cannot yet be definitely asserted, inasmuch as it is possible that a slight reaction might still have appeared at a later date. But, on the other hand, we can be certain that in two cases in which such consecutive brother and sister matings had taken place the individuality differentials of brothers had not yet reached identity.

Summary

1. The principal results obtained in these investigations are represented in the following list of average grades expressing the reactions of the hosts against transplants. These reactions depend upon the constitution of the individuality differentials of the respective fam-

ilies used for transplantation, and represent the relationship between the differentials.

Autotransplantation, grade 6.
Homoiotransplantation, grade 1.6.
Transplantation within inbred families, grade 5.5.
Multiple transplantation within inbred families, grade 5.15.
Successive transplantations within inbred families, grade 4.8 (?) (few experiments).
Transplantation from brother to brother in non-inbred families: grade 3.35.

Both combined: 5.84 (5.91)

Transplantation from brother to brother in inbred families: grade 5.87 (5.92).
Transplantation from brother to brother, hybrids of inbred families: grade 5.78 (5.89).

Transplantation from component families to hybrid between inbred families: grade 5.7.
Transplantation from hybrid between inbred families to component families: grade 3.24.

2. Within the different inbred families the individuality differentials have reached a very great similarity. The resemblance of the individuality differentials among members of an inbred family which are not closely related is much greater than that among brothers in non-inbred families.

3. There is an identity of the individuality differentials. A complete loss of individuality has not yet been reached within the inbred families. In general the individuality differentials within an inbred family seem to approach more nearly identity the greater the number of generations of brother and sister matings they had in common and the smaller the number of generations since they split off into sidelines of generations of brother and sister matings. On the whole, brothers and sisters seem to have reached identity of individuality differentials after nineteen to twenty generations of continuous brother and sister matings; at least no reaction was elicited in the host on the part of the transplant within the range of time used in our experiments. However, in a few cases there was observed a lack of complete identity of individuality differentials even under these conditions. It is probable that within certain families the homo-

geneity of individuality differentials of the various members of the family is greater than within other families. It is also probable that the average individuality differential of a certain inbred family may differ more from the average individuality differentials of various other inbred families than these differ from each other.

On this basis it would be possible to explain the stronger reactions which tissues of family 13 call forth when they are transplanted into other families, as well as the strong reaction of family 13 serving as host . In addition it is possible that certain strange genes call forth a more intense reaction than other strange genes, or that a host possessing certain genes is able to react more intensely to genes not represented in its own individuality differential.

4. In general the number of strange genes (and perhaps also the intensity of the strangeness of the composing genes) in the individuality differential of the transplanted tissue determines the severity of the reaction of the host against the transplant. The presence of strange genes in the individuality differential of the host; or expressed differently, the absence of certain genes in the transplant or the presence of double genes in the transplant, does not call forth a reaction in the host. On this basis, we can understand the difference between the results of the transplantation from component strain to hybrid, and of the reciprocal transplantation, as compared to the severity of reaction observed after the exchange of tissues within the same inbred family.

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THE HISTOGENESIS OF URINARY CASTS *

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The histogenesis of renal casts has for many years interested clinicians and clinical pathologists and much work has been done in investigating the problem. Some authors are largely speculative.[1, 2] One [3] explains the origin of casts on the basis of abnormal surface tension phenomena while others content themselves with broad generalities. Some years ago R. M. Smith [4] in an admirable paper reviewed the literature to date and attributed the origin of urinary casts to the gradual fusion of desquamated epithelium in the lumen of the tubules. He presented photomicrographs to show the gradual coalescence of this necrotic mass as it passes down to the collecting portion of the kidney. No doubt this is true in certain cases, especially in acute nephritis, but the explanation would hardly seem adequate in chronic nephritis, where even in the presence of many casts there may be little or no desquamation of renal epithelium.

While studying the rats' kidneys rendered slightly nephropathic by long continued feeding of very high protein diets, certain cells were observed to be "budding" or extruding some portion of their substance into the lumen of the tubule. A careful examination of the sections revealed what appears to be the origin of at least some types of casts, and at the same time, of the circular reticulum of the kidney.

The first change to be seen (Fig. 1) is a swelling or vacuolization of the inner portion of the cells lining the convoluted tubules. The cell becomes edematous at its inner end. As the edema increases the cell swells still further and there is formed a very striking globular or pear-shaped bud (Figs. 2, 3 and 4) connected with the main cell body by a more or less attenuated stalk. Extending into this bud, especially along its periphery, cytoplasmic material may be seen. Occasionally a disrupted nucleus is present in the bud. Under the influence of whatever forces are at play, this bud is constricted off at its base and comes to lie free in the lumen of the tubule in the form of a globe several times the diameter of the cell nucleus (Fig. 6). These

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globular bodies stain deeply with phosphotungstic acid, and constitute the familiar circular reticulum of the kidney so often seen in kidney sections. The true origin of this circular reticulum has not, I believe, been appreciated before now. The globular bodies are at times nearly devoid of cytoplasmic material. More frequently, however, they contain, particularly about their periphery, masses of deeply staining material derived from the parent cell.

Presumably under the influence of the urine, which in chronic nephritis is liable to be more acid than normal, these globular bodies partially disintegrate and the coarse masses of deeply staining material become compressed about clear circular areas once marked by the limiting membrane of the reticulum (Fig. 7). The cytoplasmic granules become finer, more densely massed together, and stain less intensely with phosphotungstic acid and more intensely with eosin. Evidences of the original arrangement can, however, still be seen (Fig. 8).

This comminuting process continues (Figs. 8 and 9) and the whole aggregate now resembles the form of the true cast though the surface is still granular, and the vacant circular areas can still be seen (Fig. 9). Finally the mass becomes truly homogeneous and stains very deeply with eosin. This is the immediate precursor of the true cast as seen in sections. It is probable that blood and epithelial cells may be caught in this coagulum as it passes down the tubules, and thus various types of casts are formed.

The different stages can be followed in succession from the earliest buds which are formed mainly in the upper portions of the convoluted tubules, through the various stages of degeneration of the circular reticulum, to the gradual comminution and coalescence of the granular débris which occurs in the loops of Henle and the collecting tubules, to the formation of a homogeneous cast.

This same process has been traced in human kidneys in cases of chronic nephritis. The swelling of the cells, which characterizes the earliest stage; and the later stages beginning with the circular reticulum to the formation of casts can be easily found, but the actual budding of the cells is infrequently seen. Only very careful examination under an oil immersion lens will reveal their presence. Typical buds have, however, been found in human tissue fixed very shortly after death.

It is not to be thought that this explanation is necessarily correct

for all urinary casts. There may well be other modes of formation, especially in acute nephritis, but it would seem to offer an adequate and logical explanation at least in certain cases.

By virtue of what pathologic change in the cell or its environment the primary swelling and budding take place is a matter for conjecture. But the process is analogous to similar phenomena which have been observed by Chambers and others when single cells outside the body have been injured, and the process may well be looked upon as merely an expression of injury on the part of the renal cells. That such is indeed the case is further evidenced by the fact that similar if not identical buddings have been found in the cells lining the uterine glands during pregnancy (Fig. 5), and a pathologic picture indistinguishable from the circular reticulum has been found in the adrenal gland in certain pathologic conditions. It may well be supposed that under the influence of changes in cell environment such phenomena may occur in widely different organs, but that only in the kidney is the subsequent history of the reticulum that of development into a cast.

SUMMARY

Evidence is presented that at least certain casts in chronic nephritis are formed by the coalescence of granules found in the circular reticulum of the kidney, and that this reticulum is in turn formed by an abnormal budding of the renal cells. This process is not peculiar to the kidney, at least so far as the initial stages are concerned, for it has been seen in other organs under varied conditions. The reaction is to be interpreted as due to an abnormal environment about the cells involved.

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DESCRIPTION OF PLATES

PLATE 80

FIG. 1. Cell of convoluted tubule showing the initial swelling and edema at the tip. × 3000.

FIG. 2. Very early bud from renal cell. Note masses of cytoplasm carried into the bud. × 3000.

FIG. 3. Later stage of bud-constriction at base. × 3000.

FIG. 4. Constricting process nearly complete. × 3000.

PLATE 81

FIG. 5. Budding cell in gland of pregnant uterus. Note the nucleus passing into the bud. × 2000.

FIG. 6. Circular reticulum of kidney. × 1000.

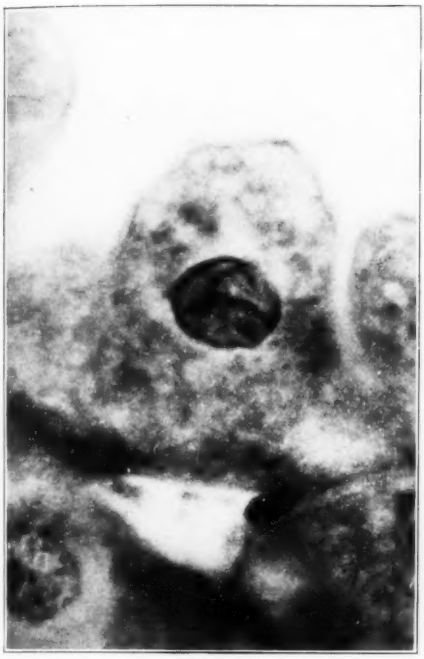
FIG. 7. Dissolution of the reticulum with the coalescence of its granules. × 1000.

PLATE 82

FIG. 8. Further comminution of the granular débris from the reticulum. × 1000.

FIG. 9. Granular mass lying in the lumen of tubule. Nearly homogeneous. Stains intensely with eosin. × 1000.

FIG. 10. Cast lying free. Homogeneous mass with circular areas still visible. × 500.



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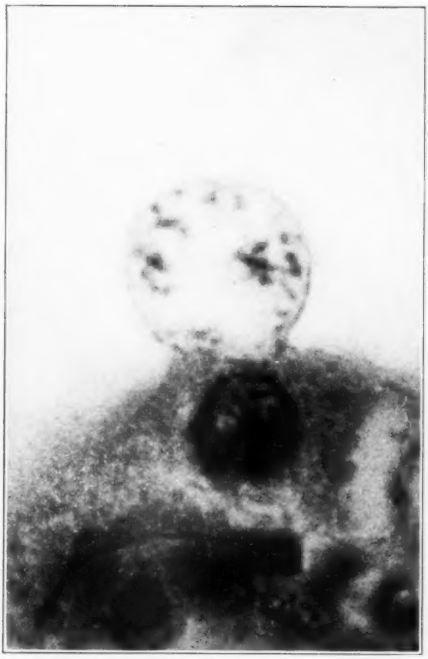

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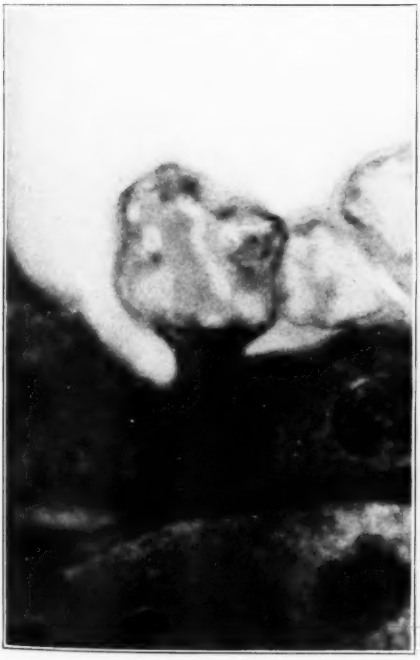

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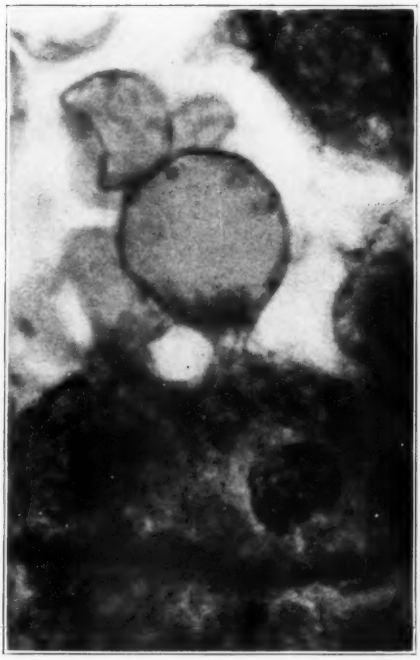

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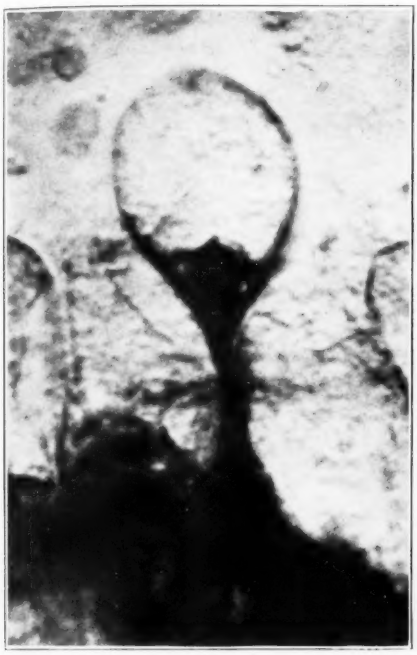

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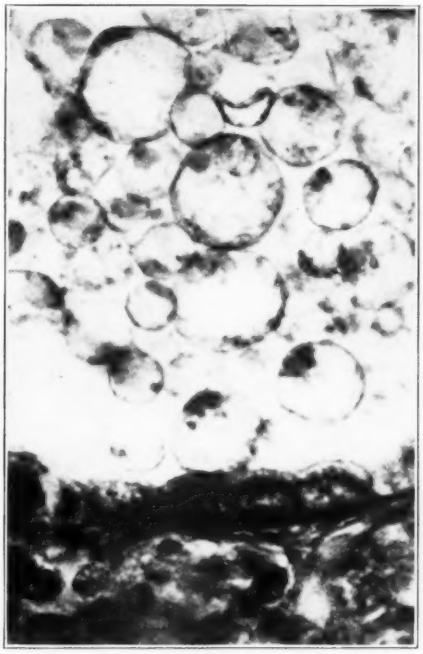

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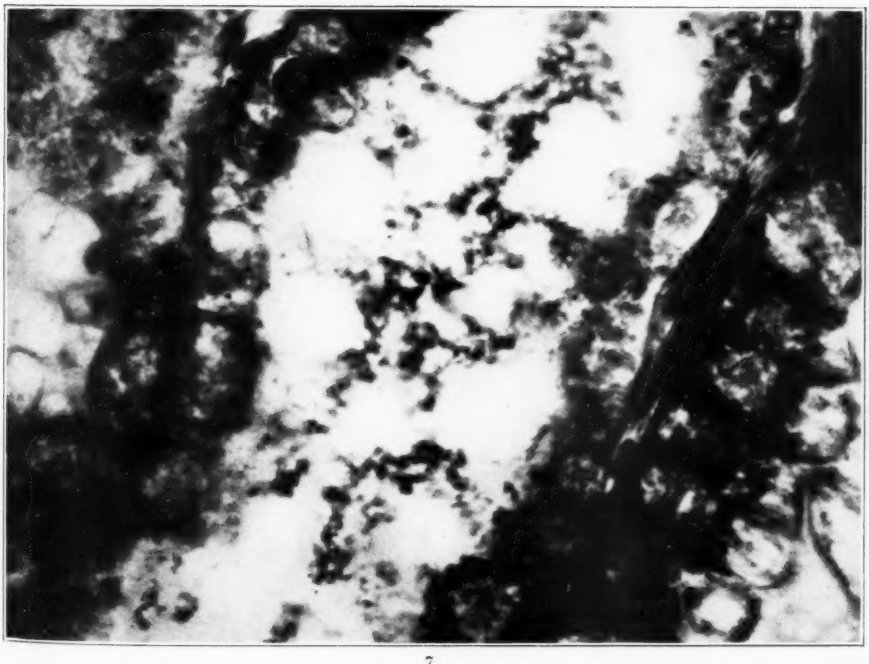

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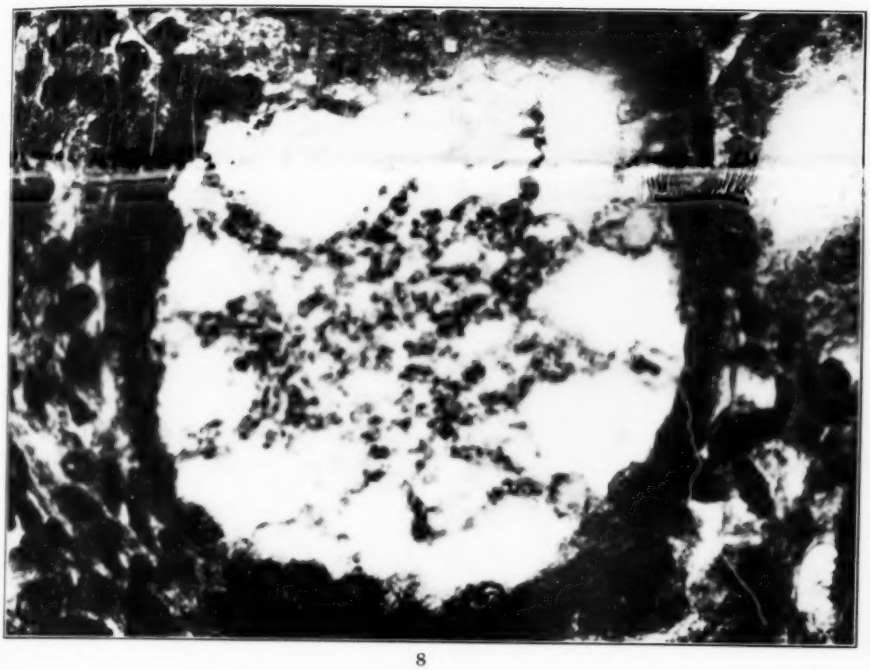

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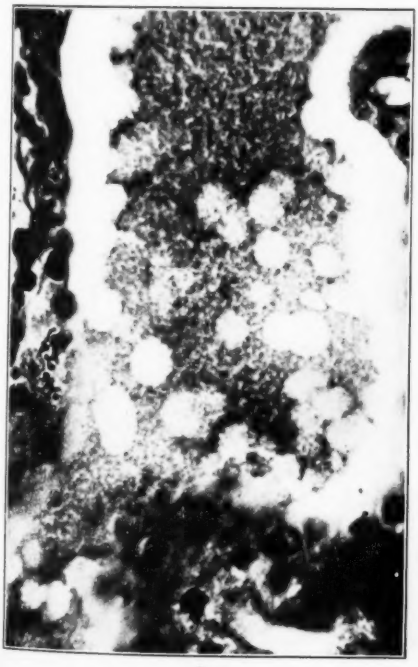

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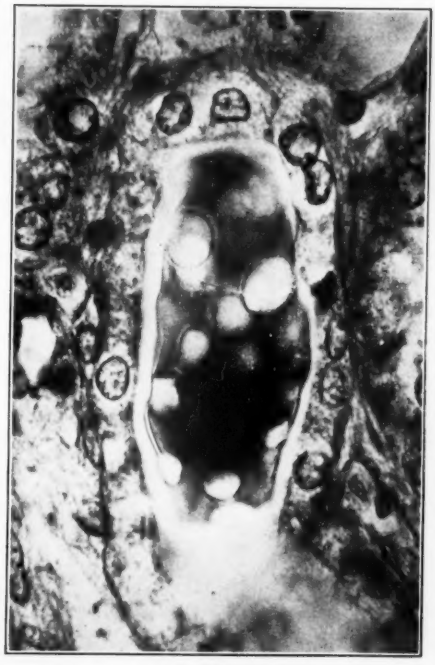

10